THYROID HORMONE DEIODINATION

PROEFSCHRIFT

ter verkrijging van de graad van doctor in de Geneeskunde
aan de Erasmus Universiteit Rotterdam
op gezag van Rector Magnificus Prof.Dr. J. Sperna Weiland
en volgens besluit van het College van Dekanen.

De openbare verdediging zal plaatsvinden op
woensdag 22 oktober 1980 des namiddags
te 2.00 uur

door

THEOFILUS JOHANNES VISSER

geboren te Rotterdam

Aan Rian,
Ellen en Renske.

CONTENTS

   I. T.J. Visser, I. van der Does-Tobé, R. Docter and G. Hennemann:
      Conversion of thyroxine into triiodothyronine by rat liver homogenate.
      Biochem. J. 150, 489-493 (1975).
  II. T.J. Visser, I. van der Does-Tobé, R. Docter and G. Hennemann:
      Subcellular localization of a rat liver enzyme converting thyroxine
      into triiodothyronine and possible involvement of essential thiol
      groups.
      Biochem. J. 157, 479-482 (1976).
 III. T.J. Visser, D. Fekkes, R. Docter and G. Hennemann:
      Kinetics of enzymic reductive deiodination of iodothyronines; effect
      of pH.
      Biochem. J. 179, 489-495 (1979).
  IV. T.J. Visser:
      Mechanism of action of iodothyronine 5'-deiodinase.
      Biochim. Biophys. Acta 569, 302-308 (1979).
   V. T.J. Visser:
      Mechanism of inhibition of iodothyronine 5'-deiodinase by thioureylenes
      and sulfite.
      Biochim. Biophys. Acta 611, 371-378 (1980).
  VI. T.J. Visser and E. van Overmeeren:
      Substrate requirement for inactivation of iodothyronine 5'-deiodinase
      activity by thiouracil.
      (submitted for publication).

VOORWOORD

Het onderzoek dat resulteerde in de bewerking van dit proefschrift werd
verricht op de afdeling Inwendige Geneeskunde III, tevens afdeling voor
Klinische Endocrinologie en Stofwisselingsziekten (hoofd: Prof.Dr. J.C. Birken-
häger), van het Academisch Ziekenhuis Dijkzigt te Rotterdam. Velen hebben mij
gesteund bij de uitvoering van de experimenten en het schrijven van dit proef-
schrift. Met name wil ik danken:
- Mijn promotor, Jorg Hennemann, voor de stimulerende discussies, zijn belang-
  stelling voor de voortgang van mijn onderzoek en de vrijheid die hij mij liet
  bij het bepalen van de onderzoekslijn;
- De co-referenten, Wim Hülsmann en Henk van der Molen, voor hun uiterst waarde-
  volle kommentaren op mijn manuscript;
- Roel Docter, voor zijn adviezen en zorg voor de technische aspekten van mijn
  werk, met name de ontwikkeling en uitvoering van de radioimmunoassays;
- Durk Fekkes, Eric Krenning, Steven Lamberts, Marten Otten en Paul Wilson, voor
  het genoegen met hen te discussiëren over en samen te werken aan het hier
  beschreven onderzoek;
- Jasper Scholte, voor zijn adviezen betreffende de subcellulaire fraktionering;
- De medewerkers van het laboratorium, voor de goede sfeer en de plezierige
  samenwerking; In het bijzonder Ineke van der Does en Ellen van Overmeeren voor
  de vaardige hulp bij de uitvoering van de experimenten met betrekking tot dit
  proefschrift;
- Corry Boot, voor het accuraat en snel uittypen van de manuscripten en de on-
  misbare assistentie bij de bewerking van dit proefschrift;
- De medewerkers van de grafische afdeling van de Audiovisuele Dienst voor de
  zorgvuldige uitvoering van de figuren.

LIST OF ABBREVIATIONS

DIT	3,5-diiodotyrosine
DTE	dithioerythritol
DTT	dithiothreitol
E-I	enzyme-iodine complex with undefined oxidation state
E-SH	enzyme with free sulfhydryl group
E-SI	enzyme-sulfenyl iodide complex
GSH	reduced glutathione
GSSG	oxidized glutathione
MCR	metabolic clearance rate
MIT	3-(mono)iodotyrosine
M-SH	methimazole; 2-mercapto-1-methylimidazole
NEM	N-ethylmaleimide
PR	production rate
PTU	5- or 6-propyl-2-thiouracil
R-SH	reduced cofactor
R-S-S-R	oxidized cofactor
rT_3	reverse T_3; 3,3',5'-triiodothyronine
T_0	thyronine
3- or $3'-T_1$	3- or 3'-iodothyronine
3,5-, 3,3'- or $3',5'-T_2$	3,5-, 3,3'- or 3',5'-diiodothyronine
T_3	3,3',5-triiodothyronine
T_4	thyroxine; 3,3',5,5'-tetraiodothyronine
Tetrac	3,3',5,5'-tetraiodothyroacetic acid
TI_n	iodothyronine
TBG	T_4-binding globulin
TBPA	T_4-binding prealbumin
TRH	TSH-releasing hormone
Triac	3,3',5-triiodothyroacetic acid
TSH	thyroid-stimulating hormone; thyrotropin
TU	2-thiouracil (also: U-SH)
U-SH	2-thiouracil (also: TU)

SCOPE OF THE THESIS

The enzymatic deiodination of thyroid hormone is an important process since it concerns - among other things - the regulation of thyromimetic activity at the site of the target organ. To understand the mechanism of this regulation it is necessary to have a detailed knowledge of the mode of action of the enzyme(s) involved in the metabolism of thyroid hormone. My investigations of the deiodination of iodothyronines at the subcellular level, forming the basis of this thesis, are described in the appendix papers. It is not intended to deal in extenso with the technical aspects of my studies in the preceeding chapters. Rather it will be attempted to give a general review of the literature including - with some emphasis - my own work.

Though not directly related to the subject of this thesis, the biosynthesis of thyroid hormone in the thyroid gland is treated in the first chapter. This is done because of possible similarities between thyroid hormone iodination and deiodination pathways, which are suggested by the finding that some drugs inhibit both processes. In the same chapter the relationship between iodothyronine structure and biological potency is described to illustrate that indeed deiodination has a dramatic effect on the activity of thyroid hormone. Besides deiodination, other pathways of metabolism are also considered.

The second chapter concerns the in vivo investigation of thyroid hormone deiodination under physiological and pathological conditions. This includes the effects of internal and external factors which affect deiodination, such as dietary intake, drugs, stress and illness. Since much work has been done to find an explanation for the effect of calorie restriction on deiodination at the molecular level, the role of the diet is emphasized. This appears particularly important since nutritional status must be considered to contribute to the change in thyroid hormone metabolism found in other situations, for example in systemic illness.

The in vitro observations of the enzymatic deiodination of thyroid hormone are described in chapter 3. A distinction has been made between (early) reports on the analysis of iodide production using chromatography, and (more recent) studies dealing with the detection of specific metabolites, often by means of radioimmunoassay. My investigations which belong to the latter category are presented in the appendix papers.

In the last chapter an attempt is made to relate the in vivo findings (described in chapter 2) to the characteristic features of the enzymatic

reactions as revealed by the in vitro experiments (described in chapter 3). Especially the role of glutathione is emphasized with respect to changes in thyroid hormone metabolism during fasting.

1. GENERAL INTRODUCTION

1.1. Thyroid hormone biosynthesis

The principal secretory products of the thyroid gland are thyroxine
(3,3',5,5'-tetraiodothyronine, T_4) and 3,3',5-triiodothyronine (T_3). Under
physiological conditions and sufficient iodine intake they are synthesized
in the human gland in a molar ratio of about 20:1. This is much higher than
the ratio of their blood production rates (approximately 3:1), indicating
an additional source for circulating T_3. It is now well established that the
extra-thyroidal monodeiodination of T_4 at the 5'-position (5'-deiodination)
is a major route of T_3 production. For the isomer, 3,3',5'-triiodothyronine
(reverse T_3, rT_3), peripheral production by monodeiodination of T_4 at the
5-position (5-deiodination) is even more important. Thyroidal secretion of
rT_3 and probably also of the lower substituted iodothyronines is almost
negligible. Thus, in normal persons, total production rates for T_4, T_3 and
rT_3 amount to about 115, 45 and 30 nmol/day, where thyroidal secretion
accounts for 100, 20 and 6%, respectively (Chopra et al, 1978a; see Table
1.1.).

Table 1.1. CONTRIBUTIONS OF THYROIDAL SECRETION AND PERIPHERAL PRODUCTION
TO TOTAL PRODUCTION RATES OF T_4, T_3 AND rT_3[a].

Iodothyronine	Total production	Thyroidal secretion (nmol/day per 70 kg body weight (%))	Peripheral production
T_4	115	115 (100)	–
T_3	45	9 (20)	36 (80)
rT_3	30	2 (6)	28 (94)

[a] Data from Chopra, 1976; Chopra et al, 1978a (see also section 2.2.).

Thyroxine is produced by coupling of two diiodotyrosine residues within
the thyroglobulin molecule. Thyroglobulin is a high molecular weight
($\sim$660 000 dalton) glycoprotein. Its tyrosyl residues are iodinated by a
process which requires the presence of iodide, hydrogen peroxide and thyroid
peroxidase (for a review, see DeGroot and Niepomniszcze, 1977). For the
mechanism of enzymatic iodination two hypotheses have been put forward. Both
models agree in that iodination does not involve the generation of a free
species of oxidized iodide, which subsequently reacts with tyrosyl moieties

14

in thyroglobulin. Rather a ternary complex is formed composed of oxidized iodide, thyroglobulin and peroxidase. The first model implies the oxidation of iodide in the presence of H_2O_2 to the iodinium ion (I^+), and the enzyme-I^+ complex formed is thought to be the active species in the iodination of tyrosine residues. The second model implies a radical reaction. Abstraction of an electron from iodide and of a hydrogen radical from tyrosine yields two radical species, I· and Tyr·, which combine under formation of an iodotyrosyl residue.
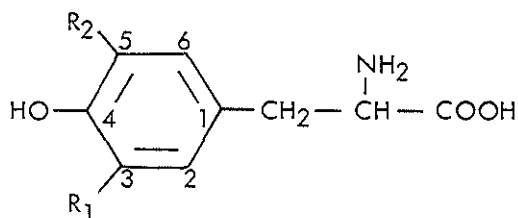
Compound	R_1	R_2
Tyrosine (Tyr)	H	H
3-Iodotyrosine (MIT)	I	H
3,5-Diiodotyrosine (DIT)	I	I
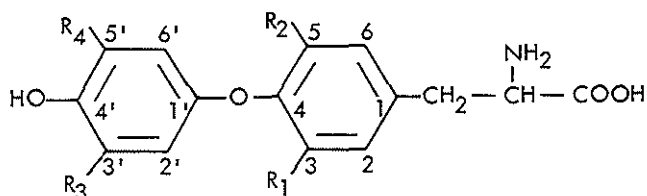
Compound	R_1	R_2	R_3	R_4
Thyronine (T_0)	H	H	H	H
3-Iodothyronine (3-T_1)	I	H	H	H
3'-Iodothyronine (3'-T_1)	H	H	I	H
3,5-Diiodothyronine (3,5-T_2)	I	I	H	H
3,3'-Diiodothyronine (3,3'-T_2)	I	H	I	H
3',5'-Diiodothyronine (3',5'-T_2)	H	H	I	I
3,3',5-Triiodothyronine (T_3)	I	I	I	H
3,3',5'-Triiodothyronine (rT_3)	I	H	I	I
3,3',5,5'-Tetraiodothyronine (T_4) (thyroxine)	I	I	I	I

Fig. 1.1. Structures of tyrosine, thyronine and iodine-substituted derivatives.

Iodination by either model yields 3-(mono)iodotyrosine (MIT), which by repetition of the sequence is converted into 3,5-diiodotyrosine (DIT). These residues are still contained within the thyroglobulin backbone. Addition of two DIT moieties by a process which is not fully understood, but which is probably also catalysed by the peroxidase, results in the formation of T_4. Apparently, production of T_3 and rT_3 by combination of MIT and DIT residues as well as the addition of two MIT's to form 3,3'-diiodothyronine (3,3'-T_2) are less preferred. (For structures, see Fig. 1.1.). Coupling, therefore, largely yields T_4 molecules still attached by their analine side chain to the remaining of the thyroglobulin molecule. They are released from the protein by the action of lysosomal proteases.

The relative uniqueness of thyroidal mechanisms in the oxidation of iodide and its subsequent incorporation suggests that thyroid hormone synthesis may be blocked specifically by intervention with these reactions. With regard to the subject of this thesis the effects of thioureylenes on thyroid activity are worth mentioning. Two members of this family of compounds, methimazole and propylthiouracil (PTU; for structures, see Fig. 1.2), are frequently used in the treatment of hyperthyroidism. They block thyroid hormone synthesis by inhibition of thyroid peroxidase. This results in an impairment of both the iodination and coupling reactions. In vivo as well as in vitro investigations have shown that methimazole is about 10 times as active as PTU (Taurog, 1976). Studies by Cunningham (1964) and Jirousek (1968) have shown that thioureylenes react specifically with protein-sulfenyl iodides. This has resulted in the hypothesis (Jirousek and Cunningham, 1968; Maloof et al, 1969) that iodination of thyroid hormone by thyroid peroxidase involves the formation of an intermediate enzyme-sulfenyl iodide (E-SI) complex.
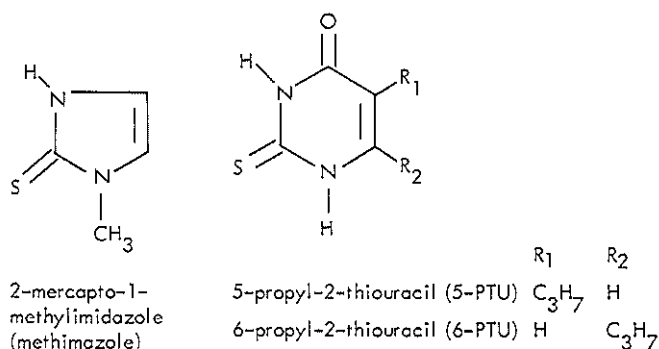

Fig. 1.2. Structures of methimazole, 5- and 6-propylthiouracil.

It has, however, been observed that thioureylenes also inhibit peroxidase activity in the absence of I^- such as in the guaiacol oxidation assay (Taurog, 1976).

The mechanism of action of these antithyroid drugs appears to be complex and depends on the concentration of the drug relative to that of iodide. In the presence of high I^- concentrations the inhibitor is extensively oxidized and inhibition is only transient, whereas at low levels of I^- little or no oxidation takes place and inhibition is persistent (Taurog, 1976; Davidson et al, 1978, 1979; Edelhoch et al, 1979). In the first case inhibition is believed to be due to competition of the drug with tyrosine for the active E-I species (the oxidation state of iodine in this complex is not defined; see above). In the second situation the inhibitor prevents this species from being formed. Reaction of thioureylenes with the E-I intermediate could result in the formation of the sulfenyl iodide (-SI) form of the drug and subsequently of other forms with oxidized sulfur, such as the disulfide (-S-S-), sulfenic (-SOH), sulfinic (-SO$_2$H) and sulfonic (-SO$_3$H) acid (Morris and Hager, 1966; Davidson et al, 1979; Lindsay et al, 1979).
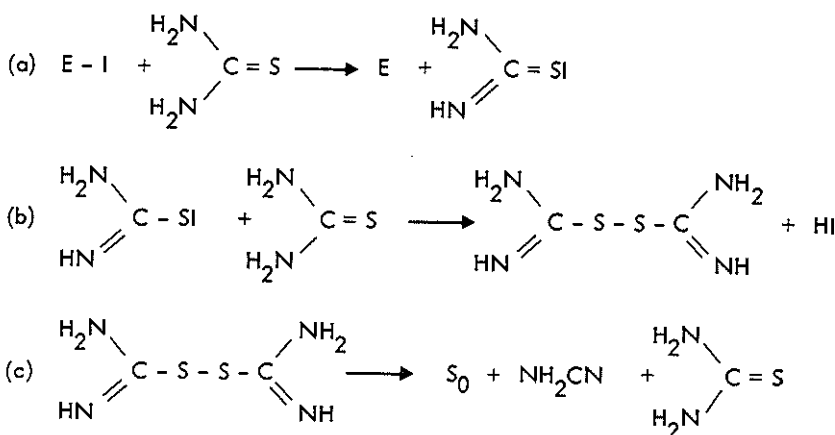
Fig. 1.3. Formation of formamidine sulfenyl iodide (a), formamidine disulfide (b) and cyanamide (c) from thiourea by thyroid peroxidase-iodine (E-I) complex.

The above mechanisms do only partly hold for thiourea. In the absence of iodide this compound is not inhibitory. In the presence of iodide the disulfide is formed (via the sulfenyl iodide), which decomposes to cyanamide (Fig. 1.3). The latter gives rise to an irreversible inactivation of the peroxidase. Inhibition of peroxidase activity by thiourea is, therefore,

twofold. Firstly, by competition for the E-I intermediate and, secondly, by virtue of its conversion to cyanamide (Davidson et al, 1979).

The generation of the sulfenyl iodide form of the goitrogens would also be difficult to reconcile with the formation of an E-SI complex as an intermediate in the iodination process. In that case one would expect an enzyme-thioureylene mixed disulfide to be formed (Cunningham, 1964).

Besides its inhibition of thyroid hormone synthesis PTU also has a pronounced effect on peripheral thyroid hormone deiodination, which property is not shared with methimazole. Results from in vitro experiments have led us to suggest that enzymatic deiodination does involve the formation of an intermediate E-SI complex (see chapter 3).

1.2 Thyroid hormone action; structure-activity relationship

Thyroid hormone is a major factor in the regulation of the resting metabolic rate. The measurement of oxygen consumption has been used as a test for thyromimetic activity of thyroid hormone analogues. Another test on which structure-activity relationships may be based is the prevention of goitre in experimental animals induced by thyroid-blocking agents (e.g. PTU). In this goitre prevention test the activity to inhibit the release of thyrotropin (negative feedback) is measured. These and other in vivo bio-assays. are blurred in the sense that the metabolism of a substance may be a major determinant in the response it elicits. This is of particular importance in the estimation of the effect of T_4, where the metabolically more potent T_3 is produced (for a review, see Jorgensen, 1976).

Recent studies have provided strong evidence that the first event in the initiation of the response to thyroid hormone is the binding to a specific nuclear receptor (Oppenheimer and Dillmann, 1978; Samuels, 1978; Latham et al, 1978). This leads to increased transcription rates and synthesis of proteins involved in the metabolic response to thyroid hormone. This sequence of events is similar to that reported for several steroid hormones, except that in case of thyroid hormone no prior binding to cytosolic receptors is required for binding at the nucleus (Docter et al, 1976). Of all compounds tested so far, binding to the nuclear receptor has been associated with a metabolic effect. The affinity for the receptor may therefore be taken as a measure for biological potency of thyroid hormone analogues.

A correlation of the in vivo activity of thyromimetic compounds with their affinity for the receptor may be obscured by differences in metabolism, in binding to serum proteins, in cellular uptake and in binding to intra-

cellular sites other than the receptor. Nevertheless, Koerner et al (1975) have demonstrated for a variety of thyronine derivatives such a correlation to exist. In Table 1.2. the relative in vivo potency of the various iodothyronines is compared with their relative affinity for the liver nuclear receptor. For comparison the relative affinities for human T_4-binding globulin (TBG) are also given. Of the naturally circulating iodothyronines only T_4 and T_3 appear to have significant biological activity, where T_3 is about 5 times as active as T_4. Since approximately 30% of T_4 is converted into T_3, any in vivo effect of T_4 may be accounted for by its transformation into T_3. Thyroxine may, therefore, be regarded as a prohormone with little or no intrinsic metabolic activity. Not only the low affinity of T_4 for the receptor, but also the finding that 90% of the endogenous thyroid hormone-receptor complexes contain T_3, while in the remaining 10% T_4 is the ligand (Surks and Oppenheimer, 1977), are in agreement with this concept.

Table 1.2. BIOLOGICAL ACTIVITY OF THYRONINE DERIVATIVES AND THEIR AFFINITY FOR PIG LIVER NUCLEAR RECEPTOR AND HUMAN TBG RELATIVE TO T_4 (%)

Compound	Biological activity		Affinity	
	Antigoiter[a]	O_2-consumption[a]	Receptor[b]	TBG[c]
T_4	100	100	100	100
T_3	850	500	900	9
rT_3	<0.2	<0.1	3	38
3,5-T_2	7	5	3	0.07
3,3'-T_2	0.5	<0.1	14	1.3
3',5'-T_2	<0.01	<0.01	0.01	0.1
3-T_1	<0.01	<0.01	−	0.05
3'-T_1	<0.01	<0.01	−	0.025
T_0	−	−	−	0

Data from [a]Jorgensen (1976); [b]Smith et al (1980); [c]Snyder et al (1976).

1.3 Pathways of thyroid hormone metabolism

The following reactions have been recognized in the metabolism of T_4: i) deiodination, ii) oxidative deamination, iii) conjugation and iv) ether bond cleavage (Fig. 1.4). Of these, deiodination is the most important pathway, accounting for at least 60% of T_4 turnover in humans. Similar fractions of T_4 are converted into T_3 and rT_3. However, deiodination is not a random
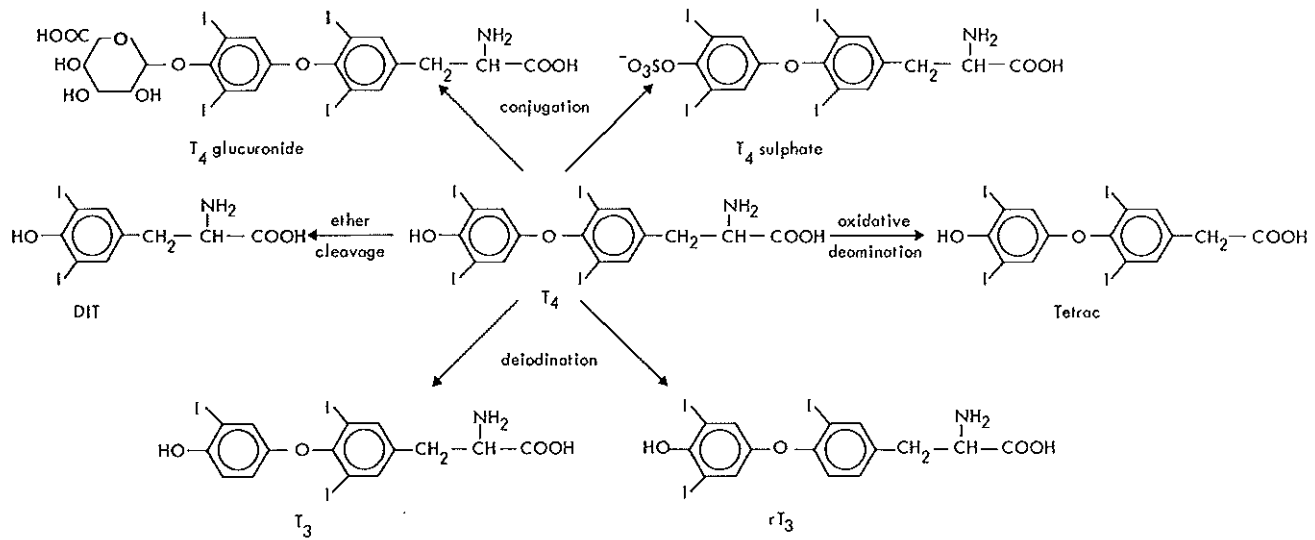
Fig. 1.4. Pathways of T$_4$ metabolism

process as has previously been suggested (Surks and Oppenheimer, 1971), but production of T_3 and rT_3 may vary independently. Both T_3 and rT_3 undergo further deiodination. The sequential deiodination of T_4 in peripheral tissues (Fig. 1.5) is thought to be mediated by two enzymes, i.e. iodothyronine 5- and 5'-deiodinase (Schimmel and Utiger, 1977; Visser, 1978).
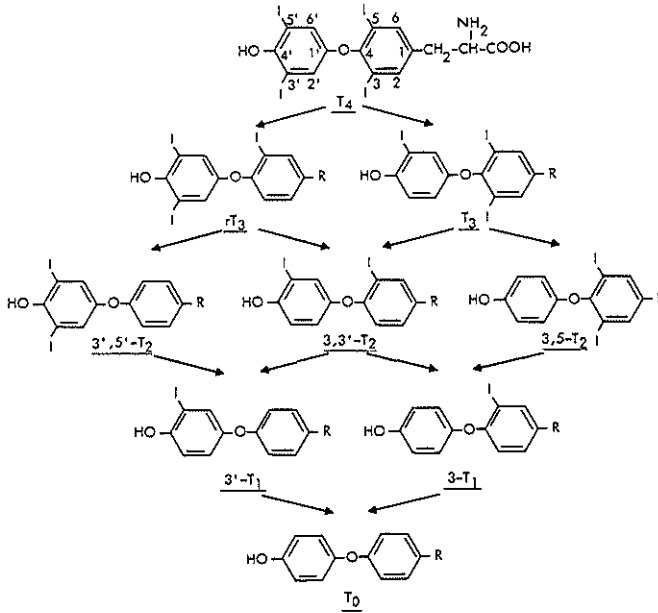

Fig. 1.5. Sequential 5-(↗) and 5'-deiodination (↘) of T_4 (note that positions 3 and 5 are equivalent as are positions 3' and 5')

The alanine side chain of iodothyronines is subject to the action of deaminating and transaminating enzymes. Oxidative deamination occurs in several tissues and leads via pyruvic acid intermediates to the formation of acetic acid derivatives (Van Middlesworth, 1974). In this way 3,3',5,5'-tetraiodothyroacetic acid (tetrac) and 3,3',5-triiodothyroacetic acid (triac) are formed. There is some controversy concerning the concentrations of these compounds in the circulation but these are certainly much lower than those of T_4 and T_3. Of interest is the high biological activity of triac in several test systems and its high affinity for the nuclear receptor (Goslings et al, 1976). The occurence of tetraiodothyroformic acid in rat liver has been reported, which is also produced via the pyruvic acid analogue (Ramsden et al, 1974). Thyroid hormone may take part in transamination reactions with

enzymes in rat liver and kidney cytoplasm, which show preference for T_3 above T_4. Surprisingly, the activity of these enzymes is stimulated by treatment of the animals with rT_3 (Fishman et al, 1977). The extent to which degradation of the alanine side chain contributes to the elimination of thyroid hormone remains to be elucidated but is probably of minor importance (Pittman et al, 1980).

Sulfate and glucuronide conjugates of thyroid hormone are formed by the action of enzymes located mainly in liver but also in kidneys (Van Middlesworth, 1974). The conjugates lack biological potency and are excreted via the bile. Some investigators have suggested that there is an "entero-hepatic" circulation of these conjugates, which would involve hydrolysis and subsequently reabsorption into the portal vene. The existence of such a cycle has, however, been contested by others. Assuming that faecal excretion of radioactivity after administration of labelled T_4 is a measure of conjugation, it has been shown that in humans 10-25% and in rats a somewhat greater fraction of T_4 is eliminated via this route. The remainder is excreted as iodide into the urine, which is representative for the deiodination process.

There is much controversy about the occurrence and possible physiological significance of ether bond cleavage as a pathway for thyroid hormone degradation (Van Middlesworth, 1974). Early studies of the in vitro deiodination of radioiodine-labelled T_4 were hampered by non-specific reactions leading to products other than T_3 and rT_3. In these reactions, which even occurred in boiled tissue preparations, iodide was produced concomitant with destruction of the thyronine structure. DIT was also produced in these circumstances (see section 3.2.). Recently, the attention of investigators has again been attracted to the possible occurrence of ether link cleavage. Balsam and Ingbar (1978) have detected the formation of DIT in rat liver homogenate in the presence of the catalase inhibitor 3-amino-1,2,4-triazole. This would indicate that this reaction may be catalysed by a peroxidase, which is in agreement with previous observations. The possible involvement of a peroxidative mechanism was also suggested from studies by Burger and Alliod (1977), who showed that this reaction is induced in leucocytes by phagocytosis. Be it as it may, the consensus is that hydrolysis of the ether bond is only a very minor pathway of T_4 degradation in the organism.

1.4. Conclusions

The thyroid secretes mainly T_4, a small amount of T_3 and a negligible amount of rT_3. The major part of T_3 and virtually all of rT_3 is produced

peripherally. Iodination of tyrosine residues in thyroglobulin and the sub-
sequent coupling of iodotyrosines to iodothyronines are both mediated by
thyroid peroxidase. The activity of this enzyme is inhibited by thioureylenes
(e.g. methimazole and PTU). The formation of an enzyme-sulfenyl iodide complex
as the active species in the iodination process appears unlikely. It is more
likely that iodination involves the production of radicals.

In several test systems the biological potency of T_3 is much higher
than that of T_4, while rT_3 is devoid of activity. Most, if not all, of the
effect by T_4 can be accounted for by its conversion into T_3. Besides the de-
iodination, iodothyronines are metabolised by conjugation to sulfates and
glucuronides, by oxidative deamination and possibly to a very minor extent
by ether bond cleavage. Of these pathways peripheral deiodination is the
most important, accounting for at least 60% of T_4 turnover. Thus, the biologic-
al effect of thyroid hormone is largely determined at the level of peripheral
tissues. Conversion of T_4 into T_3 may be regarded as an activation step,
whereas by conversion into rT_3 the hormone is irreversibly inactivated.

2.   THYROID HORMONE DEIODINATION – IN VIVO OBSERVATIONS

2.1. Introduction

    3,3',5-Triiodothyronine has been identified in thyroid extracts and plasma
by Roche, Michel and coworkers and by Gross and Pitt-Rivers (Gross and Pitt-
Rivers, 1952, 1953; Roche et al, 1952; Roche and Michel, 1954). Soon after its
isolation it was suggested (Pitt-Rivers et al, 1955) that at least part of T_3
was produced by peripheral deiodination of T_4, which could not be confirmed in a
subsequent study (Lassiter and Stanbury, 1958). However, reports on the stepwise
deiodination of T_4 have continued to appear since then. These studies also in-
cluded the possible formation from T_4 of iodothyronines other than T_3 after
Roche et al (1956a) had shown the presence of rT_3 and $3,3'-T_2$ in thyroglobulin.
Besides the demonstration of the formation of these compounds by isolated tissue
preparations (see chapter 3) several in vivo observations were also in line with
the concept that deiodination of T_4 is an important pathway for the production
of T_3, rT_3 and lower substituted iodothyronines. Especially noteworthy in this
respect are the studies of Flock and collaborators, who reported on the product-
ion of $3,3'-T_2$ from T_3 (Flock et al, 1960a,b), of rT_3 and $3,3'-T_2$ from T_4 (Flock
et al, 1961), of $3,3'-T_2$ and $3'-T_1$ from rT_3 and of $3'-T_1$ from $3,3'-T_2$ (Flock et
al, 1963) in dogs. The products were present in conjugated form in bile, plasma
and urine of normal and hepatectomized animals, although deiodination was con-
siderably slower in the latter group. Production of $3,3'-T_2$ from T_3 in rats had
also been reported by Roche et al (1956b).
    Despite these reports this matter has been controversial for many years.
This was mainly due to the lack of sensitive methods for the measurement of the
metabolites, forcing the investigators to rely on the use of radioactive isotopes
and cumbersome chromatographic techniques. A frequently encountered problem was
the spontaneous deiodination during chromatography. These techniques have im-
proved in recent years and radioactive $3,3'-T_2$ and $3'-T_1$, and $3',5'-T_2$, $3,3'-T_2$
and $3'-T_1$, together with their sulfo- and glucuroconjugates, have been isolated
from plasma after administration to humans of T_3 and rT_3, respectively, labelled
with radioactive iodine in the phenolic ring (Rudolph et al, 1978; Sakurada et
al, 1978).
    Measurement of plasma levels of the several metabolites had to await the
development of more specific techniques. In case of T_3 some progress had been
made by adaption of the competitive protein binding assay for T_4, in which chro-
matography, however, was still a critical step (Sterling et al, 1969). A major
breakthrough was obtained by the introduction of specific radioimmunoassays for

T_3 (Gharib et al, 1971) and more recently for rT_3 (Chopra, 1974), 3,3',T_2 (Wu et al, 1976), 3',5'-T_2 (Burman et al, 1978), 3,5-T_2 (Meinhold and Schürnbrand, 1978) and 3'-T_1 (Smallridge et al, 1979).

2.2. Deiodination under physiological conditions

There is general agreement in the literature concerning normal circulating levels of T_3 in humans (Chopra et al, 1978a). Less certainty exists about concentrations of rT_3 in the plasma of normal subjects. This is, among other things, caused by the impurity of the rT_3 standards used in several laboratories and by the cross-reactivity of endogenous T_4 in the rT_3 radioimmunoassay (Premachandra, 1978; Mathur et al, 1979; Meinhold and Visser, 1980). Even greater differences in serum levels of 3,5-T_2, 3,3'-T_2 and 3',5'-T_2 in normal individuals have been reported. In case of the radioimmunoassay of serum 3,3'-T_2 it has been suggested that part of the discrepancies is due to cross-reactivity by endogenous T_3 (Visser et al, 1978b).

Calculation of the daily production of a hormone is generally carried out by multiplying its plasma level with the metabolic clearance rate (MCR). The latter is an estimate of the elimination rate of the hormone and may be derived by several techniques. Preferably this should be done by noncompartmental analysis after a single injection of radioactive hormone or by the constant infusion technique (Cavalieri et al, 1971; Oppenheimer et al, 1975).

Table 2.1. MEAN SERUM CONCENTRATIONS, METABOLIC CLEARANCE RATES (MCR) AND PRO-
DUCTION RATES (PR) OF SEVERAL IODOTHYRONINES IN NORMAL HUMAN SUBJECTS

Iodothyronine	Serum concentration (nmol/1)	MCR (1/day)	PR (nmol/day)
T_4	110[a]	1.05[b]	115
T_3	1.7-2.0[c]	20-26[c]	34-52
rT_3	0.22-0.92[d]	82-108[c]	18-99
3,5-T_2	0.011-0.14[e,f]	--	--
3,3'-T_2	<0.018-0.32[g]	560-930[h-j]	<10-300
3',5'-T_2	0.012-0.12[i,k-m]	160-300[i,m]	2-36
3'-T_1	<0.06[n]	--	--

References: [a]Visser et al, 1975b; [b]Oppenheimer et al, 1975; [c]Chopra et al, 1978a; [d]Premachandra, 1978; [e]Engler et al, 1979; [f]Maciel et al, 1979a; [g]Visser et al, 1978c; [h]Gavin et al, 1978; [i]Geola et al, 1979; [j]Galeazzi and Burger, 1980; [k]Burman et al, 1978; [l]Engler and Burger, 1978; [m]Faber et al, 1979a; [n]Smallridge et al, 1979.

Mean normal plasma levels, MCR and production rates (PR) for the various iodothyronines as reported from several laboratories are listed in Table 2.1. Large variations are observed, especially for rT_3 and lower substituted iodothyronines, most of which appear to stem from uncertainties in normal plasma concentrations. If the lower reported values for serum rT_3 (20 ng/100 ml; 0.3 nmol/l) are considered to be true, total PR of this metabolite would amount to approximately 20 µg (30 nmol)/day. For T_3 and T_4 these values are approximately 30 µg (45 nmol)/day and 90 µg (115 nmol)/day, respectively (standardized to 70 kg body weight).

Thyroidal secretion rates for T_3 and rT_3 have been calculated assuming that their secretion (relative to T_4) is proportional to their content in thyroglobulin. Estimations of T_3/T_4 and rT_3/T_4 ratios in normally iodinated thyroglobulin are 0.05-0.1 (Chopra et al, 1978a) and approximately 0.015 (Chopra, 1976), respectively, from which secretion rates of around 6 µg (9nmol) T_3 and 1 µg (2 nmol) rT_3 per day, respectively, may be calculated. It therefore appears that direct secretion by the thyroid accounts for about 20 and 6% of the daily PR of T_3 and rT_3, respectively, the remainder being derived from peripheral conversion. Based on similar considerations, Abrams and Larsen (1973) have calculated - using estimates of T_3 and T_4 MCR by Schwartz et al (1971) - that in rats on a normal iodine diet, 70% of T_3 PR is derived from peripheral monodeiodination of T_4. Caution should be applied to the interpretation of these data since it has been shown that part of T_4 may be converted into T_3 and rT_3 before secretion (Laurberg, 1980). Moreover, stimulation of the thyroid (Laurberg, 1980) and iodine deficiency (Abrams and Larsen, 1973; Stevenson et al, 1974) lead to a preferential secretion of T_3.

Nevertheless, the above results are in agreement with other approaches to assess the importance of thyroidal and peripheral mechanisms in the production of iodothyronines. Firstly, similar figures were calculated by comparison of serum T_3 levels in T_4-substituted athyreotic or hypothyroid humans (Braverman et al, 1970, 1973; Surks et al, 1973; Nomura et al, 1975) and rats (Larsen and Frumess, 1977), with those in euthyroid subjects. Normal serum concentrations and turnover rates of rT_3 have also been measured in T_4-substituted subjects (Gavin et al, 1977). Secondly, estimates have been based on the quantitation of radioactive T_3 in serum of humans (Sterling et al, 1970; Pittman et al, 1971; Inada et al, 1975) and rats (Zimmerman et al, 1978; Boonnamsiri, 1979) after the administration of labelled T_4. Both kind of studies have yielded values for the fractional conversion rate of T_4 into T_3 of 25-35%, and thus provided evidence that about 80% of circulating T_3 is produced by monodeiodination of T_4. Finally,

the above findings were confirmed by the assessments of arterio-venous gradients across the thyroid in humans. Westgren et al (1977a) found that T_4, T_3 and rT_3 were secreted in a ratio of 85:9:1, and similar observations have been made by Hooper et al (1978).

Less certainty exists about turnover rates of the lower substituted iodothyronines. With respect to $3,5$-T_2 only preliminary figures concerning plasma concentrations have been published. Clearance rates have as yet not been estimated. Assuming the lower reported values of 1-2 ng/100 ml (0.02-0.04 nmol/l) for mean normal serum $3,3'$-T_2 to be real, an approximate PR of 5-20 µg (10-40 nmol/l)/day may be calculated. Although most authors agree that mean normal concentrations of $3',5'$-T_2 should be around 5 ng/100 ml (0.1 nmol/l), a different figure of only 0.6 ng/100 ml (0.012 nmol/l) has been published by Engler and Burger (1978). In addition, MCR varies according to the author between 160-300 l/day. Production rate of this compound may, therefore, assume any value between 1 and 18 µg (2-36 nmol)/day.

In analogy with T_3 and rT_3 - that is, based on their content in thyroglobulin - it has been suggested (Geola et al, 1979) that the diiodothyronines are secreted by the thyroid in insignificant quantities as compared to peripheral productions. From the above it is clear that this suggestion should be considered premature. Nevertheless, several other observations (see section 2.1. and below) point to the peripheral stepwise deiodination of T_4 as an important route by which these compounds are produced. Among these are the measurements of serum levels and daily turnover rates of $3,3'$-T_2 and $3',5'$-T_2 in athyreotic and hypothyroid subjects on T_4 substitution, which were found to be comparable to those in healthy persons (Burman et al, 1978; Gavin et al, 1978). Moreover, production from their direct precursors after administration of unlabelled T_3 and rT_3 has been detected by radioimmunoassay (Wu et al, 1976; Meinhold and Schürnbrand, 1977; Chopra et al, 1978b; Geola et al, 1980; Laurberg and Weeke, 1980).

Still less is known about the PR of the monoiodothyronines and thyronine (T_0) itself. Recently, radioimmunoassays for $3'$-T_1 have been developed and serum concentrations thereof have been found to be undetectable in euthyroid subjects. Its concentration was shown to increase after administration of $3',5'$-T_2 (Smallridge et al, 1979). Production in humans of radioactive T_0 from T_4 labelled in the phenolic ring with ^{14}C or in the alanine side chain with ^3H was suggested from experiments by Pittman et al (1970). Its excretion into the urine has been measured recently by a combination of gas chromatography and mass-fragmentography (Willetts et al, 1979). The results showed that only 20% of daily T_4 production is excreted as thyronine into the urine. The fate of the remaining 80% remains to be established.
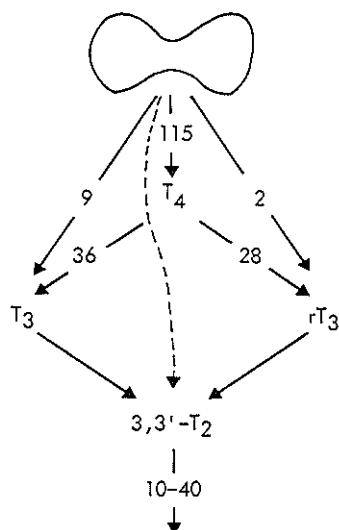
Fig. 2.1. Approximate thyroidal and peripheral production rates (nmol/day) of several iodothyronines in man.

If measurements of PR are to have any physiological significance it has to be assumed that the distribution and elimination of administered hormone is identical to that endogenously produced. For the T_4 metabolites no evidence has as yet been presented that this is the case, and attempts have been made to proof the contrary (Obregon et al, 1979). It is, therefore, not excluded that in some compartments products of T_4 metabolism are further degraded before leaving these so-called "hidden" pools. Considering the different ways of distribution between the several iodothyronines, this could mean that MCR, and therefore PR, of metabolites may be underestimated substantially. (For a summary on thyroidal and peripheral production rates of T_4, T_3, rT_3 and $3,3'-T_2$, see Fig. 2.1.).

In vivo investigations have not yielded clear indications as to the site of thyroid hormone deiodination. From observations discussed in section 2.3.3. and chapter 3 it is suggested that the liver and kidneys are important loci, but contributions from other tissues cannot be excluded. In this respect findings reported from the laboratory of Larsen are noteworthy. These workers studied the relative importance of serum T_3 and T_4 in the negative feedback on TSH secretion in rats (Larsen and Frumess, 1977). Indirect evidence was obtained for a major role of serum T_4 by the demonstration of a predominant binding of locally produced T_3 to nuclear receptors in the anterior pituitary compared with T_3 taken up from the circulation. The reverse was found if the origin of T_3 bound to nuclear receptors in the liver was investigated (Silva and Larsen, 1977, 1978; Silva et

al, 1978a; Larsen et al, 1979, 1980). Similar findings to anterior pituitary were reported for rat cerebral cortex and cerebellum (Crantz and Larsen, 1980).

2.3.   Factors affecting deiodination

2.3.1.   Inhibitors

2.3.1.1. Thiouracil derivatives

Thiourea derived compounds were introduced by Astwood (1943) as potential drugs in the treatment of hyperthyroidism (see also Astwood et al, 1945). Mainly two of these have found wide clinical application since then, i.e. methimazole and 6-PTU. It was soon recognized that besides their goitrogenic activity (see chapter 1), thiouracil (TU) derivatives also interfere with T_4 action (Andik et al, 1949; Barker et al, 1949) and metabolism (Hogness et al, 1954; Kalant et al, 1955). It has been repeatedly demonstrated that TU derivatives inhibit the response (i.e. oxygen consumption, TSH suppression, glycerol-3-phosphate dehydrogenase activity, growth hormone secretion) in rats to T_4, but not to T_3 (Jagiello and McKenzie, 1960; Stasilli et al, 1960; Escobar del Rey et al, 1962; Hsieh, 1962; Ruegamer et al, 1964, 1967, 1972; Hoffman et al, 1966; Mouriz et al, 1966; Bray and Hildreth et al, 1967; Frumess and Larsen, 1975; Hervas et al, 1976). Similar observations have been made with derivatives of 2-thiohydantoin, although these compounds may also inhibit the response to T_3 (Marx et al, 1970, 1971; Ruegamer et al, 1972; see also Tsukui et al, 1978). Generally, such an effect is not observed with methimazole although a conflicting report has appeared (Van Pilsum et al, 1973).

Thiouracil and derived compounds have been shown to slow deiodination of T_4, T_3 and other iodothyronines in rats (VanArsdel and Williams, 1956; Jones and Van Middlesworth, 1960; Hershman and Van Middlesworth, 1962; Morreale de Escobar and Escobar del Rey, 1962). This was concluded from the reduction in urinary excretion of iodide after administration of radioiodine-labelled T_4 concomitant with an augmented faecal loss of conjugates. Again, such effects are not seen with methimazole. More specifically, Flock and Bollman (1963) concluded that deiodination of T_4 at the 3' and 5' positions was decreased by TU, whereas that at the 3 and 5 positions was increased. Inhibition of the conversion of T_4 into T_3 by PTU in rats was also found by Oppenheimer et al (1972), Bernal and Escobar del Rey (1974) and Frumess and Larsen (1975). Similarly, a decrease in T_4 deiodination by PTU has been observed in man (Hershman, 1964; Furth et al, 1966). More recently, PTU administration to thyrotoxic and T_4-replaced hypothyroid patients has been shown to decrease serum T_3 levels and to increase those of rT_3 (Abuid

and Larsen, 1974; Geffner et al, 1975; Saberi et al, 1975; Westgren et al, 1977b; Laurberg and Weeke, 1978; Siersbaek-Nielsen et al, 1978). In addition, PTU has been found in humans to lower conversion of rT_3 into $3,3'-T_2$ as well as degradation of the latter (Laurberg and Weeke, 1980). Intrapituitary T_4-T_3 conversion is not affected by PTU (Larsen and Frumess, 1977; Silva and Larsen, 1978).

2.3.1.2. β-Adrenergic antagonists.

Many of the symptoms of hyperthyroidism appear to resemble the manifestations of catecholamine excess (Verhoeven, 1978). This is why cervical sympathetic chain resection has been practiced at the end of the last century as the treatment of hyperthyroidism in preference to thyroidectomy (Turner, 1974). More recently β-blocking drugs have been added to the therapeutic assortment of the physician in the abatement of thyrotoxic symptoms (Turner et al, 1965; Howitt and Rowlands, 1966; Shanks et al, 1969; Mazzaferi et al, 1976).

Several sites have been proposed for the interaction of the thyroid and the sympathetic nervous system. Despite the demonstration of sympathetic innervation of the thyroid (Melander, 1977), studies of the effects of α- and β-adrenoceptor agonists and antagonists on thyroid activity have yielded apparently conflicting results. A number of reports have indicated that several aspects of thyroid function are not affected by propranolol (Azizi et al, 1974; Wartofsky et al, 1975; Bastomsky and Lin, 1979).

It has been suggested that in hyperthyroidism there is an increased sensitivity to catecholamines. Although earlier studies have failed to show such an abnormality in experimental hyperthyroidism (McDevitt, 1976, and references therein), more recent reports have supported this suggestion (Kunos, 1977; Hashimoto and Nakashima, 1978; Fregly et al, 1979). It has also been found that thyroid hormone has a profound effect on the number and properties of α- and β-adrenergic receptor sites in some tissues, e.g. the myocardium (Ciaraldi and Marinett, 1977, 1978; Williams et al, 1977; Kempson et al, 1978; Williams and Lefkowitz, 1979).

Another possibility to account for the therapeutic merits of β-adrenoceptor antagonists, which has been investigated, is an alteration in peripheral thyroid hormone metabolism induced by these agents. In rats, adrenaline accelerates the deiodination of T_4 and T_3 (Kallman and Starr, 1959). It had been shown before that propranolol may slow the peripheral degradation of T_4 in humans (Hadden et al, 1969). It is now known that propranolol application to hyperthyroid subjects results in a decrease in serum T_3 levels and an increase in those of rT_3 often without affecting serum T_4 concentrations (Nauman et al, 1974; Murchison et al,

1976; Verhoeven et al, 1977; Wiersinga and Touber, 1977). Similar observations were made in T_4-maintained hypothyroid patients (Wiersinga and Touber, 1977; Faber et al, 1979b; Feely et al, 1979). The changes were found to be due to a decreased production rate of T_3 from T_4 and a decreased elimination rate of rT_3 (Lumholtz et al, 1978, 1979). It has been questioned whether the effects on thyroid hormone metabolism do contribute to the therapeutic value of β-adrenergic blocking drugs since it has been found that more selective $β_1$-antagonists such as practolol (Nelson and McDevitt, 1975; Murchison et al, 1976), atenolol (McDevitt and Nelson, 1978; Nillson et al, 1979) and metoprolol (Murchison et al, 1979) are as effective as propranolol in the amelioration of thyrotoxic symptoms without affecting serum T_3 levels.

2.3.1.3. Glucocorticosteroids.

Corticosteroids have been known for long to have effects on the hypothalamus-pituitary-thyroid axis in man (Ingbar and Freinkel,1956). Which site of this axis is affected appears to be a complex function of duration, dose and route of administration of the glucocorticoids. Short-term administration has indicated that these steroids reduce thyrotropin (TSH) secretion by an effect at a suprahypophyseal level (Wilber and Utiger, 1969; Nicoloff et al, 1970; Haigler et al, 1971; Singer and Nicoloff, 1973; Otsuki et al, 1973). This was concluded from - among other things - the inhibitory effect of prednisolone and other glucocorticoids on basal but not on TSH-releasing hormone (TRH)-stimulated TSH secretion. The rebound phenomenon after steroid withdrawal has been applied to the assessment of TRH reserve in patients (Singer and Nicoloff, 1973; Singer et al, 1978).

In the longer run, glucocorticosteroids appear to have an inhibitory effect on the pituitary as well (Faglia et al, 1973; Otsuki et al, 1973; Dussault, 1974; Re et al, 1976; Sowers et al, 1977). A decrease in TRH-induced TSH release has also been observed in patients with Cushing's syndrome (Otsuki et al, 1973; Kuku et al, 1975; Duick and Wahner, 1979; Visser and Lamberts, 1980). The importance of endogenous cortisol in the regulation of thyrotroph function has been demonstrated by a significant increase in serum TSH after lowering of cortisol secretion by metyrapone administration (Re et al, 1976). Also, serum TSH has been found to be increased in adrenal insufficiency (Topliss et al, 1980). Dexamethasone does not change the response of the thyroid to TSH (Vigneri et al, 1975).

The effect of corticosteroids on the hypothalamus-pituitary-thyroid axis in rats has been difficult to assess and apparently conflicting reports have

appeared. Here too, the changes observed depend on duration and dose of steroid administration (Brown and Hedge, 1973, 1974; Ranta, 1975, 1976). Nevertheless, it has been demonstrated unequivocally that physiological levels of corticosterone play a part in the regulation of pituitary sensitivity to TRH (Pamenter and Hedge, 1980).

In addition, glucocorticoid administration to humans has been shown to influence peripheral thyroid hormone metabolism. An early study by Kumar et al (1968) had suggested that glucocorticosteroids primarily alter the hepatic metabolism of T_4. This was based on an abnormal elimination of injected radioactive T_4. The rapid disappearance phase was slowed, and both fractional turnover rate and distribution volume were diminished. This seems to indicate that liver uptake of T_4 is reduced by glucocorticoids. Later studies have shown that serum T_3 is decreased and serum rT_3 is temporarily increased (Duick et al, 1974; Chopra et al, 1975; Burr et al, 1976; DeGroot and Hoye, 1976). This has been observed in normals and in T_4-substituted hypothyroid subjects. Of course, secondary to an impaired thyrotroph function, serum T_4 may also be decreased. Similar alterations have been observed in patients with Cushing's syndrome (Duick and Wahner, 1979; Visser and Lamberts, 1980) and also after administration of corticotropin to healthy persons (e.g. Westgren et al, 1977c). No effects have been observed with mineralocorticosteroids (Westgren et al, 1977c).

Finally, glucocorticoid excess has been shown to change serum binding of thyroid hormone (Oppenheimer and Werner, 1966; Gamstedt et al, 1979). Binding capacity of TBG is decreased and that of T_4-binding prealbumin (TBPA) increased such that the free fractions of T_3 and T_4 are slightly elevated.

Due to the rapid inhibition of thyroid hormone synthesis at several loci, treatment with dexamethasone in conjunction with PTU has been advocated to obtain early relief in thyroid storm (Croxson et al, 1977a). The effects of these two drugs on serum thyroid hormone levels were found to be additive. It is noteworthy that benificial effects of dexamethasone on serum T_4 were also seen in patients with Graves' disease (Williams et al, 1975). Figure 2.2. illustrates the multiple actions of glucocorticoids on the hypothalamus-pituitary-thyroid axis.

2.3.1.4. Miscellaneous

Several iodine-containing compounds, other than iodothyronines, have been reported to influence thyroid hormone deiodination in vivo (for structures, see Fig. 2.3.). Before T_3 was ever discovered, it was shown that alkyl 3,5-diiodo-4-hydroxybenzoates reduced the T_4-stimulated oxygen consumption in mice (Barker
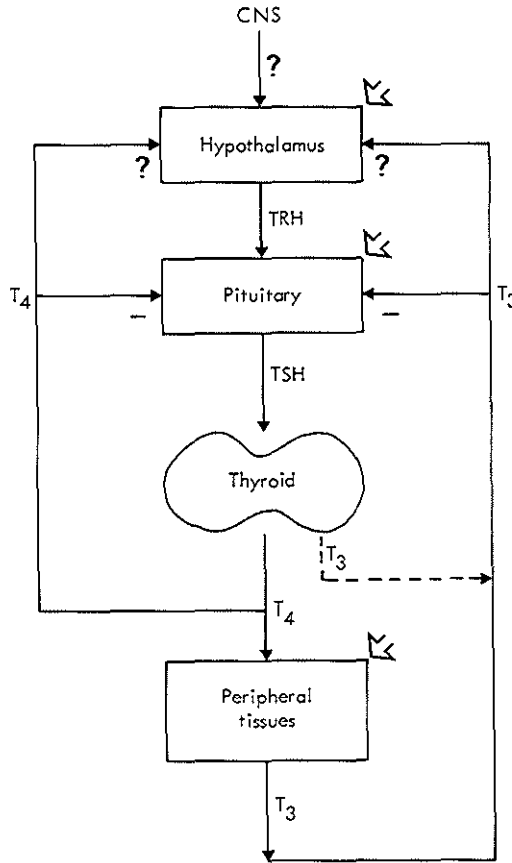
Fig. 2.2. Inhibitory effects by glucocorticosteroids (⤵) on the production of thyroid hormone.

et al, 1951; Sheahan et al, 1951). Later it was found that - as with PTU - the response to T_3 was increased (Maclagan et al, 1952). Inhibition of deiodination was thought to be the underlying mechanism of this action, as confirmed by a subsequent study (Wilkinson et al, 1954). Escobar del Rey and Morreale de Escobar (1962) and Flock and Bollman (1964) have, however, provided evidence that this is mainly a secondary effect, caused by the augmentation of the faecal excretion of T_4 conjugates.

Recently, clinical observations have been made of the inhibition of T_4-T_3 conversion and rT_3 breakdown by other iodinated compounds, e.g. amiodarone, an antiarrythmic and antianginal drug (Burger et al, 1976; Jonckheer et al, 1978), and several radiographic contrast agents having a 2,4,6-triiodoaniline structure

(Bürgi et al, 1976; Wu et al, 1978a; Suzuki et al, 1979). The latter have been used in the treatment of hyperthyroidism (Wu et al, 1978c), despite the deletorious effect of iodine administration in this condition (see also Costa, 1979; Chopra et al, 1979c). Some of these compounds also inhibit the binding of T_3 to its receptor (DeGroot and Rue, 1979). In contrast to PTU, these X-ray contrast agents do block the intrapituitary conversion of T_4 into T_3. This leads to an impairment of the TSH-suppressive effect of T_4 (Larsen et al, 1979; Suzuki et al, 1979).
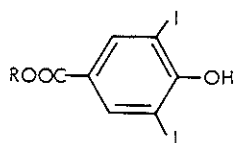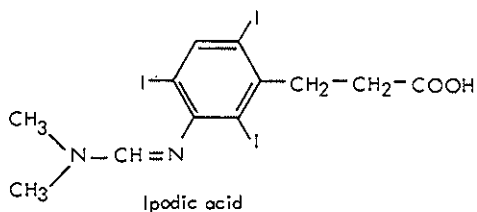
alkyl 3,5-diiodo-4-hydroxybenzoate
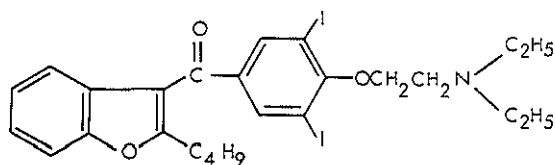
Amiodarone
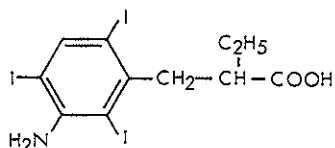
Iopanoic acid

Ipodic acid

Fig. 2.3. Structures of iodine-containing inhibitors of thyroid hormone deiodination.

In this respect it is worthwhile to mention that pharmacological doses of rT_3 blunt the metabolic response to T_4 (Pittman et al, 1959a,b; Pittman et al, 1960; Coiro et al, 1980). This is most probably due to the inhibition of the peripheral production of T_3 from T_4 (Coiro et al, 1980). The physiological significance of this effect by rT_3 is doubtful because of the huge doses needed. It has, nevertheless, been applied in the treatment of patients with Graves' disease (Benua et al, 1959).

It has been speculated that thyroid hormone may act as a neurotransmitter, and that deiodination may be mediated by tyrosine hydroxylase (Dratman, 1974; Dratman et al, 1976a,b; Dratman and Crutchfield, 1978). Indeed, T_4 degradation in rats (Dratman et al, 1976b) and the postnatal rise of serum T_3 in newborn lambs (Fisher et al, 1977) have been reported to be impaired by administration of α-methyltyrosine, a specific tyrosine hydroxylase inhibitor. Other authors, however, have been unable to demonstrate a significant effect of α-methyltyrosine administration to humans (Dvorak et al, 1978) and to rats (Pascual et al, 1979) on the conversion of T_4 into T_3.

Many drugs appear to affect serum thyroid hormone levels by interference with the binding to serum proteins. Among these is diphenylhydantoin which, in addition, appears to stimulate T_4-T_3 conversion (Cavalieri et al, 1979, and references therein). Of importance are, finally, the findings of an increased disposal of T_4 by phenobarbital treatment as a result of an enhanced biliary excretion and perhaps also increased deiodination (Oppenheimer et al, 1971; Cavalieri et al, 1973).

2.3.2. Diet

Numerous studies - in vivo as well as in vitro - have emphasized the role of nutrients in the regulation of peripheral thyroid hormone deiodination. The results have indicated that not only the amount of calories consumed but also the composition of the diet are important factors. The interest in diet induced alterations in T_4 metabolism has been aroused by the observations by Portnay et al (1974) of a selective decrease in serum T_3 concentrations in humans during starvation. These studies were extended by the demonstration of a concomitant rise in serum rT_3 levels during fasting (Vagenakis et al, 1975; Spaulding et al, 1976). Serum T_4 remained constant during the studies, and similar effects were noted in normal subjects on suppressive T_4 doses. These results were amply confirmed by others for starvation (Merimee and Fineberg, 1976; Carlson et al, 1977), semi-starvation (Grant et al, 1978; Visser et al, 1978a) and anorexia nervosa (Moshang et al, 1975; Miyai et al, 1975; Croxson and Ibbertson, 1977).

In some of these studies it was noted that in contrast to a sustained reduction of serum T_3, the rise in serum rT_3 was transient (Carlson et al, 1977; Visser et al, 1978a; see Fig. 2.4.). Overfeeding has been shown to increase serum T_3 levels (Bray et al, 1976). In subsequent investigations it has been attempted to more clearly define i) the importance of diet composition, and ii) the underlying mechanism of these changes in T_3 and rT_3 levels.
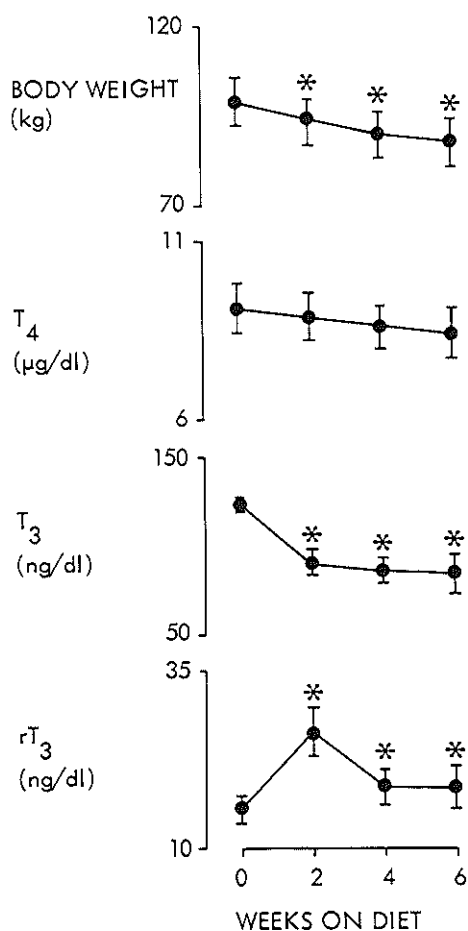

Fig. 2.4. Effects of a 300 kcal diet on body weight, serum T_4, T_3 and rT_3 (from Visser et al, 1978a).

It has been demonstrated that not so much reduction of calorie intake but rather the selective decrease in consumption of protein and carbohydrate is responsible for the changes observed. In addition, composition of the diet may affect serum T_3 and rT_3 concentrations independently. Thus, feeding obese subjects a 800 kcal diet containing no carbohydrate resulted in a lowering of serum T_3 without an effect on serum rT_3. On an isocaloric diet containing 50g of carbohydrate the concentrations of neither T_3 nor rT_3 appeared to be affected (Spaulding et al, 1976). Similar findings were reported by Azizi (1978), who showed that - after fasting - refeeding with a mixed or a 100% carbohydrate diet caused a return of serum T_3 and rT_3 to control values. In contrast, refeeding with a protein diet only restored serum rT_3 concentrations.

The importance of carbohydrate in supporting peripheral thyroid hormone deiodination has also been demonstrated by Burman et al (1979a) in a fasting-refeeding experiment, showing the efficacy of glucose and fructose in restoring serum T_3 and rT_3 levels. A requirement for the utilization of sufficient amounts of glucose to support deiodinase activity was demonstrated by the increase in serum T_3 together with unaffected T_4 and slightly decreased rT_3 levels after administration of insulin to healthy individuals (Tevaarwerk et al, 1979). Interestingly, Westgren et al (1977d) noted in fasted subjects an acute stimulation of peripheral T_3 formation by oral but not by intravenous glucose feeding. These studies, therefore, emphasize the role of hepatic glucose metabolism in the regulation of thyroid hormone deiodination (see also Saunders et al, 1978).

On the other hand, by studying the effect of normo- and hypercaloric diets, with the proportion of carbohydrate varyied between 20-80%, Davidson and Chopra (1979) concluded that the changes observed in serum T_3 were correlated best with calorie intake rather than with carbohydrate intake. Otten et al (1980) compared four diets containing 1500 kcal composed of either 100% fat, 50% fat-50% protein or 50% fat-50% carbohydrate and a control, mixed diet. It was noted that the all fat diet induced at least the same alterations in serum iodothyronine levels as did fasting. Complementing the diet with either carbohydrate or protein diminished but did not prevent the increase of serum rT_3, whereas addition of protein to the diet did not protect against the effect of fat on serum T_3 concentrations. A negative effect of fat on thyroid hormone deiodination was proposed.

It has been suggested that the decrease in T_3 seen during fasting spares muscle protein, since adjusting serum T_3 to normal (Gardner et al, 1979) or supranormal (Burman et al, 1979b) values resulted in increased muscle catabolism (see also Bray, 1977; Moore et al, 1980).

Metabolic clearance rates of iodothyronines have been measured to assess which processes are affected by a reduction of calorie intake. It was found that

the elimination of T_3 was unaltered, pointing to a decreased PR in these circumstances (Vagenakis et al, 1977; Suda et al, 1978). However, no major effect of fasting on rT_3 production was observed. Increased serum rT_3 concentrations are primarily a result of a decreased MCR (Eisenstein et al, 1978; Suda et al, 1978). Clearance rate and PR of T_4 have been found to be decreased (Grant et al, 1978; Suda et al, 1978) or normal (Vagenakis et al, 1977). A decrease may be due to diminished thyroid activity secondary to a lowering of TSH secretion (e.g. Carlson et al, 1977; Croxson et al, 1977b; Azizi, 1978; Burman et al, 1980). Overfeeding has been shown to increase both serum concentrations and MCR of T_3, irrespective of whether the extra calories were supplied by fat, carbohydrate or protein (Danforth et al, 1979). Production rates of T_4 remained constant.

The role of the diet in the regulation of peripheral T_4 deiodination has been studied less extensively in animals. A decrease in both faecal excretion and deiodination has been observed in rats during fasting (Ingbar and Galton, 1975). Consequently, serum T_4 levels were doubled within 48 hours of starvation. In contrast, fasting has been shown by Harris et al (1978a) and Kaplan and Utiger (1978a) to result in a substantial decline of both serum T_3 and T_4. Also serum TSH but not its response to TRH was decreased. Refeeding after a 4 days fast with protein or carbohydrate resulted in a rapid increase in serum T_3, whereas refeeding with fat had no effect. Refeeding after 6 days of starvation with any of the nutrients did not acutely elevate serum T_3 (Burger et al, 1980). The effect of semi-starvation in rats on serum T_3 concentrations depends on the composition of the diet. Substituting protein for carbohydrate induces a decrease in serum T_3 without affecting T_4 concentrations (Glass et al, 1978). In rabbits, starvation has been shown to induce a tenfold increase in circulating rT_3 and a 50% increase in T_4 levels, whereas those of T_3 remained constant. The MCR of T_4 did not change, that of T_3 increased and in case of rT_3 it decreased. As a result, turnover rates of all these iodothyronines were found to be increased. Since the PR of T_3 exceeded that of T_4, a substantial amount of the former appears to derive from thyroidal secretion in rabbits (Takagi et al, 1978).

An interesting observation has been the demonstration of a decrease in the number of nuclear T_3 receptors during starvation (Burman et al, 1977; DeGroot et al, 1977; Schussler and Orlando, 1978). This may be related to the finding that not only resting metabolic rate is decreased by fasting in humans (Grant et al, 1978) and in rats (Wimpfheimer et al, 1979) but also the efficacy of T_3 to stimulate it (Wimpfheimer et al, 1979). Finally, a profound decrease in serum TBG and especially TBPA was observed during caloric restriction (Moreira-Andres et al, 1980).

2.3.3. Pathological conditions

2.3.3.1. Stress

Major alterations in thyroid hormone metabolism have been observed to be induced by internal and external factors which disturb the normal functioning of the organism. Most attention has been paid to the effects of surgical stress, exposure to a cold envirnment and non-thyroidal illness, such as liver and kidney disease, myocardial infarction and infection.

Rapid elevation of rT_3 and lowering of T_3 serum concentration were observed in patients undergoing surgery (Burr et al, 1975; Brandt et al, 1976). By studying the effect of epidurial analgesia it was concluded that changes in thyroid hormone levels were independent of increased cortisol secretion (Brandt et al, 1976). A lack of correlation between cortisol and T_4 metabolism after surgery has also been reported by Presscott et al (1979). Still, Hagenfeldt et al (1979) proposed that adrenocortical activation and/or changes in the mode of nutrition may be the cause of the changes observed. A considerable reduction of serum TSH was noted by Kehlet et al (1979), but not by Hagenfeldt et al (1979).Concomitant with T_3, levels of TBPA were found to decline after surgery (Ramsden et al,1978). Qualitatively similar changes in serum thyroid hormone levels occured after total and subtotal thyroidectomy (Anderberg et al, 1979; Tamai et al, 1979; Veen, 1980). Surgery has been found to affect thyroid hormone deiodination in rabbits likewise (Ramsden et al, 1979).

Exposure of rats to cold has been known for a long time to stimulate deiodination and faecal clearance of T_4 (Kassenaar et al, 1959; Hillier et al, 1968a,b; Galton and Nisula, 1969). Conversion of T_4 into T_3 is stimulated by a cold environment (Ruegamer et al, 1964; Bernal and Escobar del Rey, 1975a,b). This is not caused by the increased food intake of the animals (Van Hardeveld et al, 1979a). It has been suggested that the sympathetic nervous system plays a role in the induction but not in the magnitude of the response to cold (Van Hardeveld et al, 1979b).

In humans, short-term (30 min) elevation to $39^{0}C$ and reduction to below $35^{0}C$ of body temperature (during and after sauna bath) failed to affect serum T_4 or T_3 levels (Tuomisto et al, 1976). In contrast, elevation of body temperature to $38.5^{0}C$ - induced by moderate exercise in a hot environment - has been shown to reduce serum T_3 and to increase serum rT_3, whereas T_4 levels remained unchanged (Epstein et al, 1979). The rapidity of the changes in T_3 suggests,however, that not only its production but also its distribution may have been altered. A recent study has indicated that similar but less pronounced changes in thyroid hormone

metabolism as seen during fasting occur during prolonged moderate exercise. A
relationship between T_4 metabolism, and uptake and utilization of free fatty
acids and glucose was suggested (O'Connell et al, 1979).

2.3.3.2. Illness

In a review, Wartofsky (1974) concluded from the existing literature that,
as a response to infection, thyroid activity is decreased secondary to inhibit-
ion of TSH release. He suggested that - as in other types of stress - this may
be mediated by cortisol. Binding of T_4 to serum proteins is usually lowered and,
unless the disease is associated with hepatic dysfunction, cellular uptake of
the hormone and therefore its fractional turnover is increased. Since then an
overwhelming amount of data has been published on the effect of acute and chronic
non-thyroidal illness on thyroid hormone metabolism.

Decreased serum T_3, increased rT_3 and normal or low T_4 levels have been
measured in a variety of sick patients and taken as indications of a reduced 5'-
deiodinase activity of the tissues (for a review, see Braverman and Vagenakis,
1979). Such an impairment of peripheral conversion may be misleading in the diag-
nosis of hyperthyroidism, since elevated T_4 levels may be accompanied by normal
serum T_3 concentrations (Britton et al, 1975; Birkhäuser et al, 1977; Engler et
al, 1978). Illness is also an important factor in the low T_3 levels measured in
elderly subjects (Davis, 1979), although also in healthy persons a negative cor-
relation between age and serum T_3, but not T_4 and rT_3 has been found (Smeulers
et al, 1979). Thus, in several studies, specific diseases have been reported to
be associated with alterations in peripheral T_4 metabolism. Among these are
febrile conditions (Rastogi et al, 1976; Wartofsky et al, 1977; Maharajan et al,
1978), myocardial infarction (Westgren et al, 1977e; Smith et al, 1978), diabetes
mellitus (Naeye et al, 1978; Saunders et al, 1978; Pittman et al, 1979a,b), liver
and kidney disease (see below).

Normal or low serum T_4, low T_3 and high rT_3 and TSH levels have been measur-
ed in patients with alcoholic cirrhosis (Copra et al, 1974; Nomura et al, 1975;
Israel et al, 1979; Walfish et al, 1979). In acute hepatitis, changes in circul-
ating thyroid hormone concentrations are less pronounced (Hepner and Chopra,
1979) and in chronic active hepatitis and primary biliary cirrhosis serum levels
of T_3 and T_4 may even increase due to enhanced binding to serum proteins (Schuss-
ler et al, 1978). Serum binding is otherwise often impaired. Interestingly,serum
T_3 was found to increase in several cirrhotic patients on treatment with PTU,
due to an improvement of liver function (Israel et al, 1979, see also Orrego et
al, 1979).

Disturbances in T_4 metabolism have also been observed in patients with renal failure. Serum T_4 is normal or decreased, serum T_3 decreased and serum TSH normal or elevated (Ramirez et al, 1976; Spector et al, 1976; Lim et al, 1977). The TSH response to TRH is usually blunted. Abnormalities were observed in patients on hemodialysis and those who were not, but amelioration was observed upon renal transplantation (Lim et al, 1977). On the average, serum T_3 and TBG levels were decreased and urinary excretion of T_3, T_4 and TBG increased in patients with nephrotic syndrome (Afrasiabi et al, 1977).

The changes in thyroid hormone metabolism in systemic illness have been shown to be due to decreased production rates of T_3 from T_4 and decreased elimination rates of rT_3 (Nomura et al, 1975; Chopra, 1976; Lim et al, 1976; Pittman et al, 1979a,b), which reverted to normal on successful treatment of the disease, e.g. diabetes mellitus (Pittman et al, 1979a,b). In general, the decrease in serum T_3 and the increase in rT_3 are related to severity of disease as estimated by several parameters such as body temperature, size of myocardial infarction, decrease in liver function, etc. (e.g. Ljunggren et al, 1977; Smith et al, 1978; Hepner and Chopra, 1979). The low serum T_4 encountered in a substantial number of sick patients may be the result of decreased binding to serum, caused by a circulating inhibitor (Chopra et al, 1979a,b). It is doubtful whether alterations in T_4 metabolism are a consequence of enhanced cortisol secretion (Kallner and Ljunggren, 1979; Ljunggren et al, 1979).

2.4. Conclusions

Several approaches have been used to assess the contributions of thyroidal secretion and peripheral conversion to the total production of T_3, rT_3 and lower substituted iodothyronines. These are i) measurement of serum concentrations of these compounds in subjects without appreciable thyroid function receiving substitution therapy with synthetic T_4, ii) quantitation of radioactive metabolites in plasma after administration of labelled T_4 under steady state conditions and iii) comparison of secretion rates with total turnover rates. For the latter, the amounts of iodothyronines secreted have been estimated knowing that thyroidal secretion accounts for all T_4 delivered to the circulation and either i) assuming that secretion of these compounds is proportional to their content in thyroglobulin or ii) by direct assessment of arterio-venous gradients across the thyroid.

These experiments have unequivocally demonstrated that a large proportion, i.e. about 80%, of circulating T_3 and virtually all of circulating rT_3 is derived from monodeiodination of T_4 in peripheral tissues. Although the origin of the

Effects:

Conditions:
- Caloric deprivation   – Drugs: Propranolol,
- Systemic illness   PTU, dexamethasone,
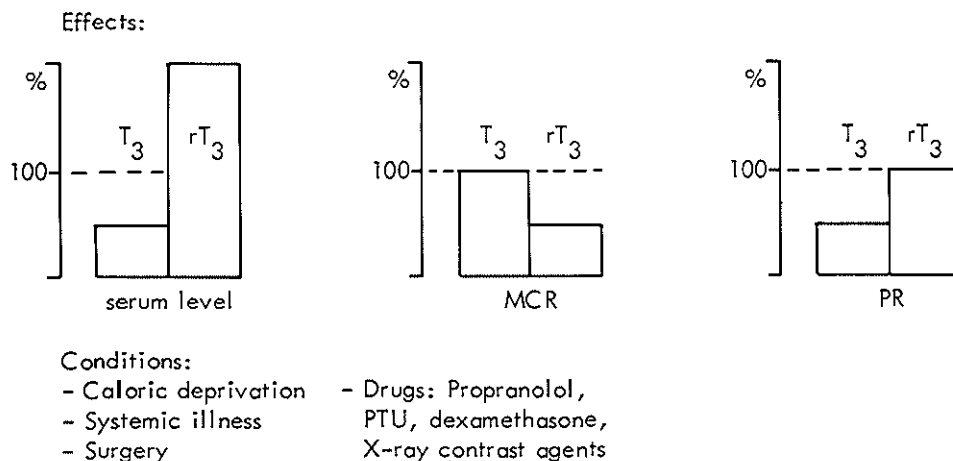- Surgery   X-ray contrast agents

Fig. 2.5. Effects of various conditions on serum levels, metabolic clearance
rate (MCR) and production rate (PR) of T_3 and rT_3 (% of control)

di- and monoiodothyronines has not been elucidated with similar conviction, it
is likely that peripheral production is a major source for these compounds.

Productions of T_3 and rT_3 from T_4 are not random processes. Under a
variety of conditions (summarized in Fig. 2.5.) conversion of T_4 into T_3 is
diminished whereas conversion into rT_3 does not change. Serum levels of rT_3
in these situations, however, increase owing to an impaired elimination. These
findings are compatible with the hypothesis that the peripheral deiodination
of T_4 is mediated by two enzymes, i.e. a 5'-deiodinase converting T_4 into T_3
and rT_3 into $3,3'-T_2$, and a 5-deiodinase converting T_4 into rT_3 and T_3 into
$3,3'-T_2$. Diminution of T_3 production and rT_3 degradation is then explained
by a selective decrease in 5'-deiodinase activity.

The in vivo observations have not yielded conclusive evidence with regard
to the site of thyroid hormone deiodination. The abnormalities found in liver
and kidney disease may indicate that these organs play an important role in
this respect. This is supported by the data obtained during the investigations
of these reactions in tissue preparations as will be discussed in the next
chapter. Further, evidence has been presented for the occurence of the con-
version of T_4 into T_3 in the pituitary and various brain regions. The contrib-
utions of these sites to the total production of T_3 is probably negligible,
notwithstanding their importance in the supply of the active hormone to local
receptors.

3.   THYROID HORMONE DEIODINATION - IN VITRO OBSERVATIONS

3.1. Introduction

The hypothesis that most of circulating T_3 is derived from monodeiodin-
ation of T_4 in peripheral tissue has appealed to many investigators. It is
not surprising then, that a lot of work has been done to reveal the principles
involved and the mechanism of this reaction. Despite these efforts, relatively
little progress has been made until recently, when in vitro studies have yield-
ed unequivocal information about the enzymatic nature of iodothyronine deiodin-
ation in tissue preparations. These investigations have already shed some
light on the question of the mechanism of these reactions and the regulation
of deiodinase activity in the organism.

The reason for this success undoubtedly is the result of the introduct-
ion of reliable and specific radioimmunological techniques to measure the
products of the deiodination reactions. Although in several studies deiodin-
ated products (e.g. T_3) have been recovered from reaction mixtures containing
tissue preparations and radioactive substrate (e.g. T_4), most of the early
studies have dealt with the production of iodide. In these latter investigat-
ions generally no specific products other than ill-defined "origin material"
- referring to its chromatographic properties - have been observed. In the
light of more recent studies, most of these results may be regarded as artific-
ial probably without physiological significance. Not only for historical reasons,
but also since they have contributed to our understanding of the chemistry
of thyroid hormone, these studies will be discussed in the next section. This
is followed by an overview of results from mainly the recent literature, which
allow a more reliable interpretation.

3.2. Degradation of iodothyronines with release of iodide

The generation of iodide on incubation of radioiodine labelled T_4 with
a variety of tissue preparations has been reported. Thus, deiodination has
been observed using the perfused rabbit (Becker and Prudden, 1959) and rat
liver (Flock and Owen, 1965), and slices, homogenates and subcellular fractions
of rat liver, kidney, skeletal muscle and brain (Table 3.1.). Also, rat thyroid
(Dawber et al, 1971) and tissues from frogs and tadpole (Dowling et al, 1964;
Yamamoto, 1964a; Galton et al, 1965) have been studied. Deiodinative activity
was associated with the particulate fractions (e.g. microsomes) of liver and
kidney homogenates (Stanbury et al, 1960; Yamamoto et al, 1960; Wynn et al,

1962; Nakagawa and Ruegamer, 1967), but with the supernatant fraction of skeletal muscle and brain (Tata, 1958, 1960; Lissitzky et al, 1961a,b).

Considerable attention has been given to the formation of the iodinated "origin material" which often accompanied deiodination (Galton and Ingbar, 1961; Plaskett, 1961; Lissitzky et al, 1961a,b; Wynn and Gibbs, 1962; Surks et al, 1969; Kozyreff et al, 1971). This material was found to contain iodine covalently bound to protein. This could be due to some form of transiodination involving the iodines of the phenolic ring, but it has also been shown to contain the intact substrate (Kozyreff et al, 1971) and breakdown products such as DIT (Plaskett, 1961; Wynn and Gibbs, 1962). It has been suggested that this type of reaction may also occur in vivo (Surks et al, 1969).

The mechanism of non-specific deiodination has not been fully clarified. From the reported characteristic features of this process (Table 3.1.), one gets the impression that distinct reactions are involved. It has been suggested (Galton and Ingbar, 1963, 1964) that under certain conditions degradation of T_4 in tissue preparations may be mediated by peroxidase, since stimulation was observed with H_2O_2 and the catalase inhibitors azide and 3-amino-1,2,4-triazole, and inhibition with catalase. Contradictory observations were made by Nakagawa and Ruegamer (1967). The role of peroxisomes has not been investigated. Discordant findings have also been reported with respect to the effects of thiols, ascorbate, p-chloromercuribenzoate and thiouracil. Breakdown of T_4 may even occur in boiled tissues. Based on different sensitivities to heat Galton and Ingbar (1966) distinguished enzymatic and non-enzymatic components of tissue homogenates. In general, stimulation is seen with flavins and Fe^{2+}, and inhibition with Hg^{2+} and cyanide. Except in brain and possibly muscle, T_4 breakdown is oxygen dependent.

Special mention must be made of the degradation of T_4 by white blood cells. Deiodination and iodoprotein formation have been observed in intact cells (Klebanoff and Green, 1973; DeRubertis, 1974; Woeber, 1976a) and in homogenates (Kurland et al, 1960; Woeber, 1976b). Deiodinative activity is associated with the granule fraction (Woeber, 1976b), and is stimulated in phagocytosing leucocytes (e.g. Klebanoff and Green, 1973). Iodination of intracellular protein was also detected in cells incubated with iodide (e.g. Klebanoff and Clark, 1977), especially after induction of phagocytosis. All these processes (i.e. iodination, de- and transiodination) are mediated by myeloperoxidase, which is located in the granules, and require H_2O_2, the production of which is stimulated by phagocytosis (e.g. Klebanoff and Green, 1973). Iodination (and subsequent killing) of ingested microorganisms has

Table 3.1. EFFECTS OF CONDITIONS ON THE DEGRADATION OF T_4 (WITH RELEASE OF IODIDE) IN TISSUE PREPARATIONS AND TISSUE FREE INCUBATIONS

	Stimulation	Inhibition	No effect
Liver and kidney	Flavins[a], Fe^{2+}[b-d], thiols[c], ascorbate[c], T_4 in vivo[m], phenobarbital in vivo[r], H_2O_2[x], azide[z], amino-triazole[z].	Anoxia[a-f], heat[a,b,f,l], Hg^{2+}[c,f,m], cyanide[a-d,f,m], thiols[m], ascorbate[m,n], EDTA[c,d,f,m], thiouracil[p], azide[d], catalase[z], p-chloro-mercuribenzoate[c,m].	Flavins[d,m], heat[j-l], thiols[d], thiouracil[b,f], H_2O_2[d,m], catalase[d,m], azide[m], p-chloromercuri-benzoate[d,f].
Skeletal muscle	Flavins[g,h], Fe^{2+}[h], H_2O_2[x], T_4 in vivo[q].	Anoxia[g], heat[g,i], Hg^{2+}[g-i], thiols[g], ascorbate[g].	Anoxia[i].
Brain		Heat[i,o], Hg^{2+}[i,o], cyanide[o].	Anoxia[i,o].
Buffer	Flavins + light + O_2[s-w], H_2O_2 + peroxidase[x,y].		

References: [a]*Yamamoto et al, 1960;* [b]*MacLagan and Reid, 1957;* [c]*Stanbury et al, 1960;* [d]*Wynn et al, 1962;* [e]*Sprott and MacLagan, 1955;* [f]*Yamazaki and Slingerland, 1959;* [g]*Lissitzky et al, 1961a,b;* [h]*Tata, 1960;* [i]*Tata, 1957;* [j]*Lissitzky et al, 1959;* [k]*Etling and Barker, 1959;* [l]*Galton and Ingbar, 1966;* [m]*Nakagawa and Ruegamer, 1967;* [n]*Yamamoto, 1964b;* [o]*Tata et al, 1957;* [p]*Braverman and Ingbar, 1962;* [q]*Tata, 1961;* [r]*Schwartz et al, 1969;* [s]*Lissitzky et al, 1961c;* [t]*Suzuki et al, 1961;* [u]*Galton and Ingbar, 1962;* [v]*Morreale de Escobar et al, 1962,1963;* [w]*Reinwein and Rall, 1965;* [x]*Galton and Ingbar, 1963;* [y]*Mayrargue-Kodja et al, 1958;* [z]*Galton and Ingbar, 1964.*

been suggested to perform the function of a host defense mechanism (Klebanoff, 1967). In sepsis, cellular uptake and metabolism of T_4 are accelerated (De-Rubertis and Woeber, 1973; see also chapter 2.3.). It has, therefore, been suggested that phagocytosing leucocytes may utilize thyroid hormone as a source of iodine for bactericidal activity (e.g. Klebanoff and Green, 1973).

In many instances, T_4 and other iodothyronines have been found to be degraded in chemically defined media (Table 3.1.). Thus, "deiodination" was observed in mixtures containing flavins in the presence of oxygen and light. Flavin dependent deiodination is stimulated by inert proteins such as fibrinogen and albumin (Morreale de Escobar et al, 1962, 1963; Reinwein and Rall, 1965) and inhibited by Cu^{2+} and Hg^{2+} (Galton and Ingbar, 1962). In this system the ether bond is split and DIT is formed (Lissitzky et al, 1961c, Suzuki et al, 1961). Deiodination also occurs in the presence of H_2O_2 or H_2O_2-generating systems, and is further stimulated by peroxidase (Mayrargue-Kodja et al, 1958; Galton and Ingbar, 1963) or chelated metal ions (Reinwein et al, 1968). Also here, the ether bond has been found to be cleaved (Mayrargue-Kodja et al, 1958).

The above findings indicate that degradation of T_4 may be observed in tissue preparations as well as tissue-free incubations, especially where conditions allow for the formation of free radical species. This degradation is an oxidative process and is characterized by iodide production, ether bond cleavage and iodination of protein (if present). Especially noteworthy in this respect are the studies by Wynn (1968) and Nakano and coworkers (Nakano et al, 1971, 1973; Ushijama et al, 1973; Suwa and Nakano, 1975). These authors have linked T_4 deiodination by microsomes with lipid peroxidation. Both lipid peroxidation and deiodination are induced by Fe^{2+}, which may be kept in the reduced state either enzymatically (NADPH dependent) or chemically (ascorbic acid dependent). Ferrous iron induces the formation of lipid, lipid alkoxy and lipid peroxy radicals, initiating a chain reaction characteristic for fatty acid autooxidation (Chow, 1979). Thyroxine interferes with the propagation of this chain reaction by reacting with the alkoxy and peroxy radicals, and may be regarded as an antioxidant in this respect (Wynn, 1968; Suwa and Nakano, 1975).

However, in the presence of peroxidase and H_2O_2, T_4 enhances lipid peroxidation (Kumar et al, 1977). The common factor in all these observations may be the ability of T_4 to form a phenoxy radical. In subsequent steps, the iodine atoms of the phenolic ring are eliminated as the radical (I·; Kumar et al, 1977) or as the anion (I$^-$; Suwa and Nakano, 1975) and the ether bond is cleaved (see Fig. 3.1).
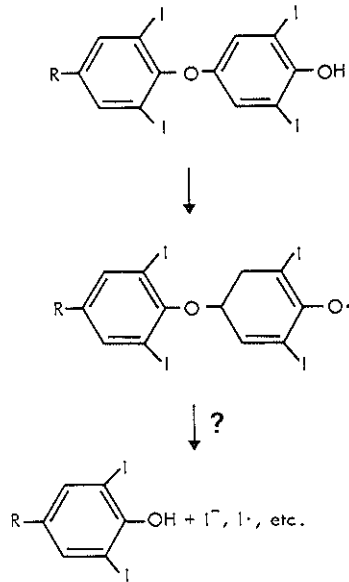
Fig. 3.1. Oxidative breakdown of T_4 via the phenoxy radical yielding DIT, iodide and other products.

Lipid peroxidation is a degenerative reaction, and the cell is well equipped with defense mechanisms which prevent damage from this process. The antioxidant potential of the cell comprises radical scavengers such as vitamin E and superoxide dismutase, while peroxide concentrations are kept at a minimum level by catalase and glutathione peroxidase (Chow, 1979). It is, therefore, likely that the type of deiodination described in this section is of limited physiological relevance, with the possible exception of T_4 degradation by leucocytes.

3.3 Production of specific metabolites

Production of radioactive T_3 from radioiodine-labelled T_4 by intact cells has been detected with the aid of chromatographic techniques. Using this method conversion has been shown to occur in several tissue preparations, e.g. rat kidney slices (Albright et al, 1954; Cruchaud et al, 1955; Balsam et al, 1978a), human kidney slices (Albright and Larson, 1959), rat liver slices (Green, 1976; Balsam et al, 1978a), perfused rat heart (Rabinowitz and Hercker, 1971), rat thyroid (Haibach, 1971; Green, 1978) and pituitary

fragments (Silva et al, 1978b; Cheron et al, 1979), human leucocytes (Kleba-noff and Green, 1973) and cell cultures of human fibroblasts (Refetoff et al, 1972), liver and kidney cells (Sterling et al, 1973) and rat pituitary tumors (Gershengorn et al, 1979). Using similar techniques, Papavasiliou et al (1977) noted conversion of rT_3 into $3,3'-T_2$ in a rat pituitary tumor in culture. Noteworthy are further studies by Sorimachi and coworkers of the deiodination of several iodothyronines in monkey hepatocarcinoma cells in culture. These cells were found to be active in the 5-deiodination of T_4, T_3, $3,3'-T_2$ and $3,5-T_2$ and the 5'-deiodination of rT_3 and $3',5'-T_2$. From the incubations $3,3'-T_2$ and $3'-T_1$ were primarily recovered as sulfates. Interestingly, no conversion of T_4 into T_3 could be observed (Sorimachi and Robbins, 1977, 1978a,b, 1979a,b; Sorimachi and Cahnmann, 1979).

Radioactive substrates and chromatographic analysis of the reaction products have also been employed in the analysis of the conversion of T_4 into T_3 in homogenates and subcellular fractions of rat kidney (e.g. Chira-seveenuprapund et al, 1978) and liver (Harris et al, 1978b; Balsam et al, 1978b).

Deiodination of unlabelled substrates (by quantitation of metabolites with radioimmunoassay) has been demonstrated to take place in various intact cell preparations. For instance, conversion of T_4 in T_3 has been detected in perfused rabbit kidney (Adlkofer et al, 1977) and rat liver (Hesch et al, 1975; Jennings et al, 1979), isolated rat hepatocytes (Hesch et al, 1975; Van Noorden et al, 1979) and renal tubules (Heyma et al, 1978), rat pituitary tumor in culture (Melmed et al, 1979) and human leucocytes (Woeber and Maddux, 1978). Production of rT_3 from T_4 by the kidney (Heyma et al, 1978) and leucocytes (Woeber and Maddux, 1978), and of $3,3'-T_2$ from rT_3 by liver cells (M.H. Otten and T.J. Visser, unpublished observations) have similarly been observed.

By far most of the observations of the enzymatic deiodination of iodo-thyronines have been made using broken cell preparations, measuring the products with the aid of specific radioimmunological techniques. Most of these studies have employed rat liver and kidney homogenates and subcellular fractions thereof. In this system the following reactions have been followed: conversion of T_4 into T_3 (e.g. Hesch et al, 1975; Visser et al, 1975a, 1976a; Hüfner and Knopfle, 1976; Chopra, 1977; Kaplan and Utiger, 1978a), conversion of T_4 into rT_3 (e.g. Cavalieri et al, 1977; Höffken et al, 1977; Hüfner et al, 1977; Visser et al, 1978c, 1979a), conversion of rT_3 and T_3 into $3,3'-T_2$ (e.g. Chopra et al, 1978c; Höffken et al, 1978a; Hüfner and Grussendorf,

1978; Visser et al, 1978c, 1979a) and conversion of $3',5'$-T_2 into $3'$-T_1 (Visser and Van Overmeeren, 1980a). $5'$-Deiodination of T_4 in rat pituitary homogenates has also been followed by radioimmunoassay of T_3 (Silva et al, 1978b; Kaplan, 1980).

3.4. Localization of deiodinase activity

There is general agreement that, irrespective of the substrate used, deiodinase activity is associated with the particulate fractions of tissue homogenates, predominantly with the microsomes (e.g. Hesch et al, 1975; Visser et al, 1976b). Deiodination by this fraction is stimulated by the addition of cytosol (Visser et al, 1976b; see Fig. 3.2.) suggesting the
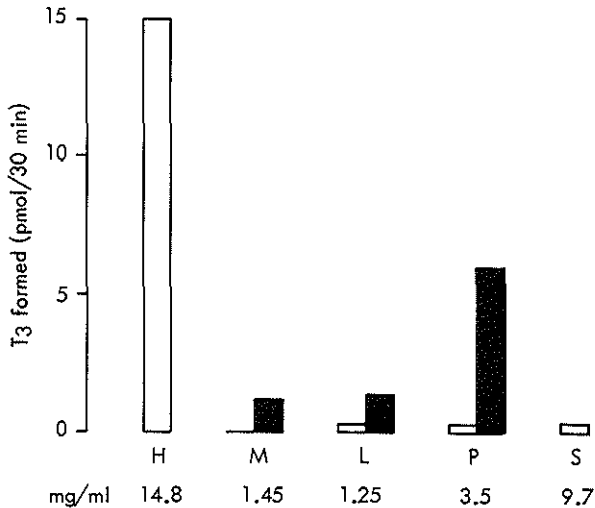

Fig. 3.2. Conversion of T_4 into T_3 by rat liver homogenate (H), mitochondrial (M), lysosomal (L), microsomal (P) and supernatant (S) fractions (□); the effect of addition of the supernatant to the particulate fractions (■). (Concentrations of fractions given as mg protein/ml; from Visser et al, 1976b).

requirement for a soluble cofactor. Highest conversion rates of T_4 into rT_3 were observed by Cavalieri et al (1977) in rat liver cytosol, but this finding may be due to a slight contamination of this fraction by microsomal protein in combination with - strangely enough - a relative lack of rT_3 $5'$-deiodinase activity (see also Fekkes et al, 1979). Conversion of T_4 into rT_3 in homogenates or microsomes is difficult to estimate due to the highly effective

breakdown of rT_3 by 5'-deiodination (Hüfner et al, 1977; Visser et al, 1978c).
More recently, investigators have attempted to define more exactly the intra-
cellular location of deiodinase activity. Leonard and Rosenberg (1978a) ob-
tained evidence for a plasma membrane location for T_4 5'-deiodinase activity
in rat kidney homogenate. The same, was also suggested by Maciel et al (1979b)
for the liver enzyme, although this was not supported by their own data. Auf
dem Brinke et al (1979, 1980) and Fekkes et al (1979) found a clear associat-
ion between deiodinase activity and marker enzymes for liver endoplasmic
reticulum. This was found to be true following both 5- and 5'-deiodinations.

3.5. Characteristic features of enzymatic deiodination

It is now well established that the deiodinations of iodothyronines are
enzymatic processes. To certain limits, deiodinase activity is linearly related
to the concentration of microsomal protein in the reaction mixture, and accumul-
ation of products is linear with time of incubation (e.g. Visser et al, 1979a).
The factors involved with the conversion of T_4 into lower substituted iodo-
thyronines are sensitive to heat. Heating tissue preparations for only 30 min
at 56°C was sufficient to destroy deiodinase activity completely (Visser et
al, 1975a). Several authors have reported on the inhibitory effect of SH
group-blocking agents (see section 3.7). Moreover, it has been demonstrated
repeatedly that 5- and 5'-deiodinase catalysed reactions obey Michaelis-Menten
kinetics, where apparent K_m values for most reactions in rat liver homogenate
or microsomes vary between 1 and 10 µM (Chopra, 1977; Hüfner et al, 1977;
Chopra et al, 1978c; Höffken et al, 1978b; Kaplan and Utiger, 1978a; Kaplan
et al, 1979; Visser et al, 1979a). However, the apparent K_m of rT_3 in the
5'-deiodinase-catalysed conversion into $3,3'-T_2$ is substantially lower, i.e.
10^{-7} - 10^{-8} M (Hüfner et al, 1977; Chopra et al, 1978c; Kaplan and Utiger,
1978a; Kaplan et al, 1979; Visser et al, 1979a). For $3',5'-T_2$ in the enzymatic
5'-deiodination into $3'-T_1$ an intermediate apparent K_m value (10^{-6} - 10^{-7} M)
has been estimated (Visser and Van Overmeeren, 1980a; D. Fekkes, E van Over-
meeren and T.J. Visser, unpublished observations). Similar estimates of K_m
values for T_4 and rT_3 have been reported for the 5'-deiodinase from rat kidney
(Chiraseveenuprapund et al, 1978; Kaplan et al, 1979). However, quite different
figures have been published for the hepatocarcinoma (Sorimachi and Robbins,
1979b) and anterior pituitary enzymes (Kaplan, 1980).

The effect of pH on the several deiodinations has been studied extensively.
In liver preparations, conversion of T_4 into T_3 has been found to be most
effective at pH 6-7 (Cavalieri et al, 1977; Chopra, 1977; Höffken et al, 1977;

Hüfner et al, 1977; Visser et al, 1978c, 1979a). In first instance, production of rT_3 from T_4 was found to be optimal at pH 9-9.5 (Höffken et al, 1977; Hüfner et al, 1977). It was, however, observed that at more physiological pH values, rT_3 was degraded too rapidly into $3,3'-T_2$ to allow its direct measurement. By following the production of $3,3'-T_2$ instead it was deduced that 5-deiodination of T_4 was most effective at pH 8 (Hüfner and Grussendorf, 1978; Visser et al, 1978c, 1979a). This value is in agreement with that found by Cavalieri et al (1977), who studied this reaction in rat liver supernatant fraction. Conversion of T_3 into $3,3'-T_2$, which is analogous to the conversion of T_4 into rT_3 (5-deiodination), has also been shown to be optimal at approximately pH 8 (Chopra et al, 1978c; Höffken et al, 1978a; Visser et al, 1978c, 1979a).
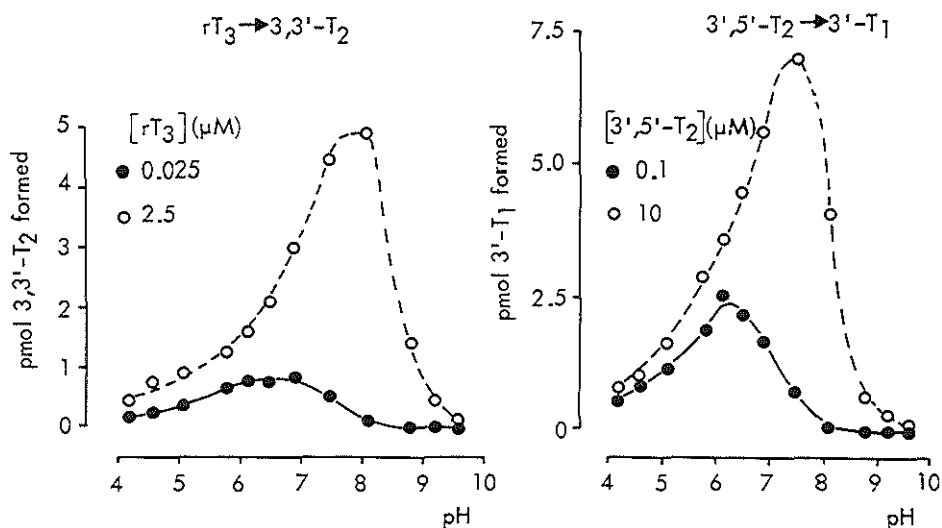

Fig. 3.3. Dependence on substrate concentration of the effect by pH on the conversion of rT_3 into $3,3'-T_2$ and of $3',5'-T_2$ into $3'-T_1$ by rat liver microsomal fraction (from Visser and Van Overmeeren, 1980a).

Large discrepancies have been reported with respect to the effect of pH on the conversion of rT_3 into $3,3'-T_2$, which is analogous to the production of T_3 from T_4 (5'-deiodination). Optimal values for pH have been reported to amount to 8-9 (Chopra et al, 1978c), approximately 8 (Hüfner and Grussendorf, 1978), 7.7 (Gavin et al, 1980a), 7.3 (Höffken et al, 1978a) and 6.5 (Visser et al, 1978c). It has, however, been shown that the effects of pH on production rates of $3,3'-T_2$ depend on the concentration of rT_3 tested (Visser et al,

1979a; Visser and Van Overmeeren, 1980a). At low substrate concentrations, reaction rate is highest around pH 6.5, whereas with high rT_3 concentrations, deiodination proceeds more rapidly at about pH 8. Strikingly similar observations have been made in the study of the conversion of $3',5'-T_2$ into $3'-T_1$, also a 5'-deiodination (Visser and Van Overmeeren, 1980a; see Fig. 3.3). As far the 5'-deiodination, the apparent K_m for rT_3 is much lower and the V_{max} much higher than the corresponding values for T_4 (Visser et al, 1979a). These kinetic parameters are different functions of pH depending on whether rT_3 or T_4 is substrate (Visser et al, 1979a; see Fig. 3.4.).
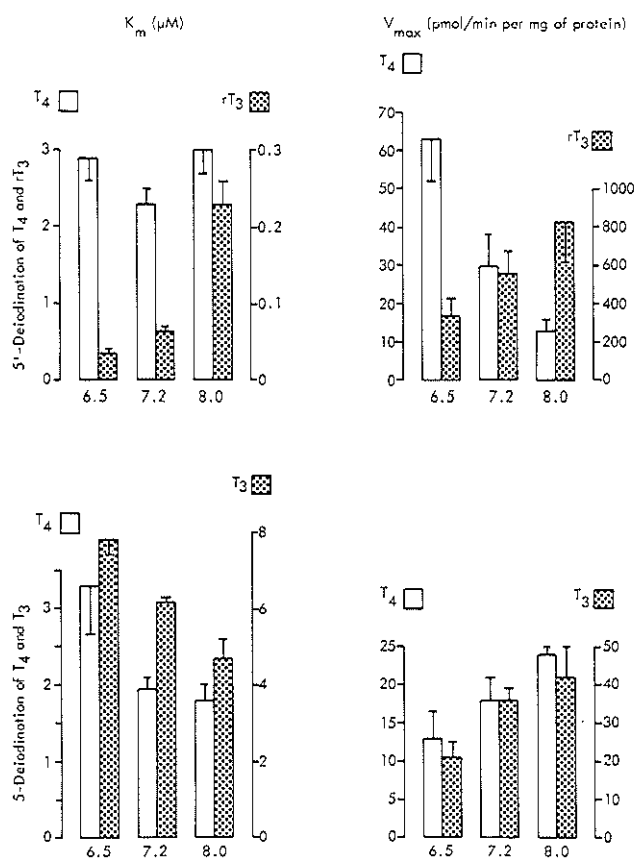

Fig. 3.4. Effect of pH on the kinetic parameters of enzymatic 5- and 5'-deiodinations (means ± S.E.M., n=3-5; from Visser et al, 1979a).

Despite these differences, evidence has been obtained that both reactions are catalysed by a single enzyme (iodothyronine 5'-deiodinase). Thus, T_4 is a competitive inhibitor of the 5'-deiodination of rT_3 (Kaplan and

Table 3.2. EFFECTS OF CONDITIONS ON ENZYMATIC DEIODINATION OF IODOTHYRONINES IN RAT LIVER AND KIDNEY PREPARATIONS

Stimulation	Inhibition	No or slight effect
Thiols[a-j], anoxia[b,k], EDTA[a,k], diphenyl-hydantoin in vivo[t], hyperthyroidism[w,x,y].	Heat[e,k,p], NEM[d,k], H_2O_2[e,k], t-butylperoxide[d], iodoacetic acid[m], silver and mercury ions[a,b,d], mercuri compounds[a,d,e], GSSG[d], diamide[d,n,o], SO_3^{2-}[r], thiouracil[j-m,p,q], dinitrophenol[g,m], X-ray contrast agents[g], dexamethasone in vivo[q,t,u], fasting[q,u,v], diabetes[u], hypothyroidism[w,x,y].	NAD(P)[a,c,k,l], NAD(P)H[a,c,k,l], EDTA[b,l], flavins[a,k], methimazole[r,s], thiourea[r,s], tyrosine[a,c,l], MIT[a,c,l], DIT[a,c,l], dinitrotyrosine[a,c,l], α-methyltyrosine[c,k,l], propranolol[k], dexamethasone[l,q], diphenylhydantoin[l], I^-[e,k,r], CN^-[e,k,r], SCN^-[r,s], N_3^-[k,l,r], Fe^{2+}[q], Fe^{3+}[q], ascorbic acid[l].

References: [a]Visser et al, 1976b; [b]Cavalieri et al, 1977; [c]Hüfner et al, 1977; [d]Chopra, 1978; [e]Höffken et al, 1978b; [f]Harris et al, 1979 ; [g]Kaplan et al, 1979; [h]Leonard and Rosenberg, 1978a; [i]Visser, 1979; [j]Visser et al, 1978c; [k]Chiraseveenuprapund et al, 1978; [l]Chopra, 1977; [m]Chopra et al, 1978c; [n]Balsam et al, 1979a; [o]Kaplan, 1979a; [p]Visser et al, 1975a; [q]Kaplan and Utiger, 1978a; [r]Visser, 1980a; [s]Leonard and Rosenberg, 1978b; [t]Hüfner and Knöpfle, 1976; [u]Balsam et al, 1978b; [v]Harris et al, 1978b; [w]Grussendorf and Hüfner, 1977; [x]Balsam et al, 1978a; [y]Kaplan and Utiger, 1978b.

Utiger, 1978a; Visser et al, 1979a) and vice versa (Chopra, 1977; Kaplan and Utiger, 1978a), where K_i and K_m values were found to be equal under various conditions. Also $3',5'-T_2$ competitively inhibits the 5'-deiodination of T_4 (Chopra et al, 1978a). Evidence may be obtained that conversion of T_4 into rT_3 and of T_3 into $3,3'-T_2$ is mediated by a separate enzyme (iodothyronine 5-deiodinase). Kinetic parameters of both conversions showed to be similar functions of pH (Visser et al, 1979a; see Fig. 3.4.). Not only the relatively low level of 5'-deiodinase activity of monkey hepatocarcinoma cells, but also the finding that the inhibitory effect of T_4 on the 5'-deiodination of rT_3 is negligible ($K_i \sim 10^{-5}$ M) compared with its potent inhibition of the 5-deiodination of $3,5-T_2$ ($K_i \sim 10^{-8}$ M), strongly suggest that separate enzymes are involved in these processes (Sorimachi and Robbins, 1979b). Based on differences in K_m values with small variations in pH, Auf dem Brinke et al (1979) suggested that more than one enzyme catalyses the conversion of T_4 into T_3. The significance of these observations remains to be established.

The characteristics of enzymatic deiodination of iodothyronines in homogenates and microsomal fractions of rat liver and kidney are summarized in Table 3.2. Converting activity is impaired in tissues from fasted and diabetic rats. Enzyme activities are decreased in hypothyroid, and increased in hyperthyroid animals. In general, concomitant changes are observed in the conversion of T_4 into T_3 and that of rT_3 into $3,3'-T_2$ (Kaplan and Utiger, 1978a,b; Kaplan et al, 1979). Properties of deiodinase activities in liver and kidney appear to be very similar (Kaplan et al, 1979). However, their regulation in vivo differs in some respects, judged from the effects of thyroid (Kaplan and Utiger, 1978b) and nutritional status (Kaplan et al, 1979). A very interesting observation is that - in contrast to peripheral tissues - deiodinase activity in the pituitary is decreased in hyperthyroidism, increased in hypothyroidism and unaffected by fasting (Cheron et al, 1979; Kaplan, 1980). Also, Sorimachi and Robbins (1978b) found that deletion of nutrients from the incubation medium had no effect on phenolic ring deiodination by hepatocarcinoma cells, whereas non-phenolic ring deiodination was even accelerated.

Addition of propranolol, α-methyltyrosine, dexamethasone or diphenylhydantoin does not influence deiodinase activities of tissue homogenates. Propranolol, in high concentrations (>290 μM), inhibits conversion of T_4 into T_3 by rat hepatocytes (Van Noorden et al, 1979), whereas lower concentrations also do not interfere with the formation of $3,3'-T_2$ from rT_3 in this system

(M.H. Otten and T.J. Visser, unpublished observations). Liver and kidney homogenates from rats pretreated with dexamethasone show decreased T_4 5'-deiodinase activities. Treatment of fetal sheep with cortisol paradoxically enhances deiodinase activity of liver and kidney (Wu et al, 1978b). Pretreatment of rats with diphenylhydantoin has a stimulatory effect (Hüfner and Knöpfle, 1976).

3.6 Cofactors

Stimulation of T_4 5'-deiodinase activity of rat liver homogenate was observed by the addition of NAD while NADH, NADP and NADPH were without effect (Visser et al, 1975a). The stimulatory effect by NAD could not be confirmed in a subsequent study (Visser et al, 1976b). A lack of effect by addition of any of these nicotinamide-adenine dinucleotides has also been reported by other authors (Chopra, 1977; Hüfner et al, 1977; Chiraseveen-uprapund et al, 1978), although NADPH may stimulate T_4-T_3 conversion in tissue preparations of fasted (Balsam et al, 1979a) and hypothyroid (Balsam et al, 1979b) animals (see chapter 4). Flavins have been shown to have no effect on enzymatic deiodination, although addition to rat liver homogenate stimulated non-enzymatic production of T_3 from T_4. This was not observed upon exclusion of light (Visser et al, 1975a).

During subcellular fractionation of rat liver homogenate T_4 5'-deiodinase was lost so that in none of the fractions obtained production of T_3 could be detected. Only on combining the particulate fractions, in particular the microsomal fraction, with the 100 000 x g supernatant converting activity was recovered (Visser et al, 1976b). This has been confirmed by other investigators (Balsam et al, 1979a,b; Kaplan, 1979a,b). These findings indicate that there is an absolute requirement for a soluble cofactor in the cytosol. In the investigation of the possible constitution of this factor it was found that cytosol could effectively be replaced by simple thiols such as dithio-threitol (DTT), 2-mercaptoethanol and 2,3-dimercaptopropanolol, and it was suggested that reduced glutathione (GSH) is the endogenous cofactor (Visser et al, 1976b; see Fig. 3.5.).

Since then a large number of reports has been published on the stimulation of deiodinase activity in various tissue preparations by mercapto compounds. Thus, thiols stimulate both 5- and 5'-deiodinase activities of liver and kidney (Table 3.2.), leucocytes (Woeber and Maddux, 1978) and hepatocarcinoma cells (Sorimachi and Robbins, 1979b). Mercapto compounds also stimulate conversion of T_4 into T_3 in pituitary tumor cells (Melmed et
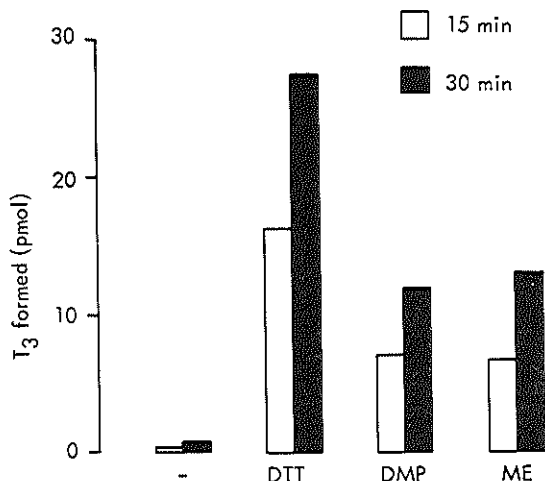
Fig. 3.5. Effects of 1 mM dithiothreitol (DTT), dimercaptopropanol (DMP) or
mercaptoethanol (ME) on the conversion of T_4 into T_3 by rat liver
microsomal fraction (from Visser et al, 1976b).

al, 1979) and pituitary homogenates (Silva et al, 1978b; Kaplan, 1980).

Dithioerythritol (DTE) and DTT are the most potent stimulators of deiodin-
ase activity, while 2-mercaptoethanol and 2,3-dimercaptopropanol are less
active, and GSH is clearly the least effective (Visser et al, 1976b; Cavalieri,
1977; Chopra, 1978).

It has recently been established that thiols are real cofactors in deiodin-
ation processes, since it has been demonstrated that there is an active part-
icipation of these compounds in these reactions. Thus, thiols act as the second
substrate in the 5'-deiodination of T_4 (Leonard and Rosenberg, 1978b) and of
rT_3 (Visser, 1979), reactions which are believed to follow a ping-pong mechanism
(see section 3.8.). Therefore, the enzymatic deiodination of iodothyronines
(TI_n) is a reduction, where the reductive equivalents are supplied by thiol
cofactors (R-SH): $TI_n + 2$ R-SH $\rightarrow TI_{n-1} + $ R-S-S-R + HI (note that TI_{n-1} contains
missing H).

3.7 Inhibitors

Two classes of inhibitors of 5- and 5'-deiodinase-catalysed reactions
have been studied in some detail. In the first category are the compounds
which react more or less specifically with sulfhydryl groups. Conversion of
T_4 into T_3 in tissue homogenates and subcellular fractions is inhibited by

addition of N-ethylmaleimide (NEM), H_2O_2, diamide, silver and mercury ions, mercury containing compounds such as p-chloromercuriphenylsulfonic acid, t-butylperoxide and oxidized glutathione (GSSG; Table 3.2.). Iodoacetic acid has been shown to inhibit formation of $3,3'-T_2$ from both T_3 and rT_3 in rat liver homogenate in a non-competitive fashion (Chopra et al, 1978c). Conversion of $3',5'-T_2$ into $3'-T_1$ is also blocked by the action of NEM on rat liver microsomes (Visser and Van Overmeeren, 1980b). After incubation with NEM, Ag^+, Hg^+ or p-chloromercuriphenylsulfonic acid, deiodinase activity was not restored by the subsequent addition of excess thiols, indicating the presence of essential cysteine residues in the enzyme(s) (Visser et al, 1976[b]; Visser and Van Overmeeren, 1980b). The non-competitive inhibition by iodoacetic acid may also point into this direction.

The second group of compounds, which mainly consists of the thiouracil (TU) derivatives, appears to react also with an essential sulfhydryl group of the enzyme, but only after this has been oxidized during the deiodination process. Notably PTU has been reported to inhibit both 5- and 5'-deiodinations in a variety of tissue preparations, i.e. either intact cells (Cruchaud et al, 1955; Larson et al, 1955; Green, 1976; Heyma et al, 1978; Woeber and Maddux, 1978; Van Noorden et al, 1979) or homogenates and subcellular fractions (Table 3.2.). Inhibition has also been observed in tissue preparations from PTU-pretreated rats (Larson et al, 1955; Leonard and Rosenberg, 1978b; Balsam et al, 1978b; Kaplan et al, 1979). Sorimachi and Robbins (1979a,b) found only phenolic ring deiodination by hepatocarcinoma cells to be inhibited by PTU, whereas deiodination of the tyrosyl ring was unaffected. Generally, inhibitory effects are observed with methimazole only at high concentrations (>1 mM), although inhibition occurred also at 10 µM in leucocytes (Woeber and Maddux, 1978). The structure-activity relationship of thioureylenes in the inhibition of 5'-deiodinase activity has been investigated, and the low activity of methimazole was found to be due to the N^1-methyl group (Visser et al, 1979b).

Inhibition by thiouracil and its derivatives is of the uncompetitive type (Chopra, 1977; Chopra et al, 1978c; Leonard and Rosenberg, 1978b; Visser, 1979), and is competitively antagonized by DTT, methimazole and thiourea (Leonard and Rosenberg, 1978b; Visser, 1979, 1980a). A similar mode of inhibition is exerted by sulfite. However, its action is additive to that of methimazole and thiourea (Visser, 1980a). It is of interest that also where the action of PTU in vitro is concerned, deiodination in the anterior pituitary has characteristics different from those in peripheral tissues. Inhibition of T_4-T_3 transformation could be detected neither in tissue fragments (Cheron et al, 1979) nor in homogenates (Kaplan, 1980).

The mutual competitive inhibition by iodothyronine substrates has been discussed in section 3.5. In addition, pretreatment of rats with large doses of rT_3 resulted in a substantial lowering of T_4-T_3 converting activity of liver homogenates (Coiro et al, 1980). Tyrosine, MIT, DIT and dinitrotyrosine do not or only weakly inhibit iodothyronine deiodinase activity (see also Larson and Albright, 1961; Sorimachi and Robbins, 1979b). The latter is a specific inhibitor of iodotyrosine deiodinase (Haibach, 1971). However, dinitrophenol does lower iodothyronine deiodinase activity, although not in hepatoma cells (Sorimachi and Robbins, 1978a). Competitive inhibition by several radiographic contrast agents of T_4-T_3 and rT_3-3,3'-T_2 converting activity of liver, kidney (Kaplan et al, 1979) and pituitary homogenates (Kaplan, 1980) has been described. Judged from their structures (Fig. 2.3.), these compounds may be regarded as substrate analogues.
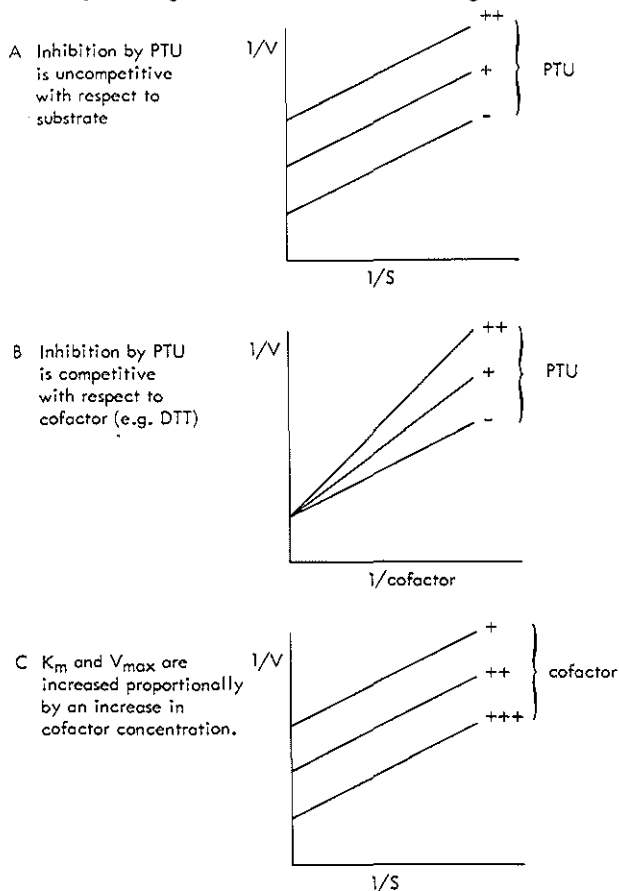

Fig. 3.6. Kinetics of enzymatic 5'-deiodination; effect of PTU and cofactor.

3.8. Possible mechanisms of deiodination

Key observations concerning the pathway of enzymatic 5'-deiodination are (see Fig. 3.6.): i) Lineweaver-Burk diagrams of iodothyronine substrate (TI_n) concentration against monodeiodinated product (TI_{n-1}) formation at various fixed concentrations of thiol compounds yield a set of parallel lines; ii) Lineweaver-Burk diagrams of TI_n concentration against TI_{n-1} formation at various fixed concentrations of TU or its derivatives yield another set of parallel lines; iii) Lineweaver-Burk diagrams of thiol concentration against TI_{n-1} formation at various fixed concentrations of TU or its derivatives yield a set of lines which intersect on the ^{1}/v axis (Leonard and Rosenberg, 1978b; Visser, 1979). These results indicate that enzymatic 5'-deiodination follows a socalled ping-pong mechanism, a reaction involving two substrates and two products, which enter and leave the enzymatic cycle sequentially (Dixon and Webb, 1979). Thus, reaction of the first substrate (A) with the enzyme (E) yields an intermediate enzyme complex (E') concomitant with the formation of the first product (P). Reaction of the intermediate with the second substrate (B) results in the generation of native enzyme and the second product (Q; see Fig. 3.7.).
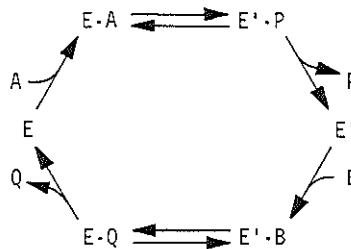

Fig. 3.7. Ping-pong mechanism of enzymatic reaction involving two substrates (A,B) and two products (P,Q).

The findings suggest that TU reacts with the same intermediate as the thiol cofactor. Since other studies (see section 3.7.) had indicated that iodothyronine-deiodinating enzymes contain at least one essential cysteine residue, it was suggested (Leonard and Rosenberg, 1978b; Visser, 1979) that the intermediate was formed by conversion of a sulfhydryl group into a higher oxidation state. It has been shown that TU reacts more or less specifically with protein-sulfenyl iodides under formation of protein-thiouracil mixed disulfides (Cunningham, 1964; Jirousek, 1968; see Fig. 3.8.).
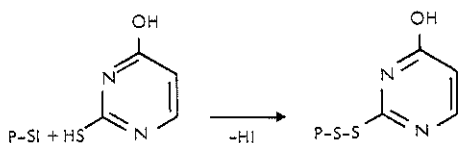
Fig. 3.8. Reaction of thiouracil with protein-sulfenyl iodide (P-SI) yielding
        a protein-thiouracil mixed disulfide (according to Cunningham, 1964).

As a working hypothesis, it has been proposed (Visser, 1979, 1980a,b)
that thyroid hormone deiodination implies the formation of a deiodinase-
sulfenyl iodide (E-SI). Such a derivative is formed by transfer of an iodinium
ion (I^+) from the substrate to a sulfhydryl group of the enzyme (E-SH). To
complete the cycle, the E-SI complex is subsequently reduced by cofactor
(R-SH). It is also subject to reaction with thiouracil (U-SH) yielding a
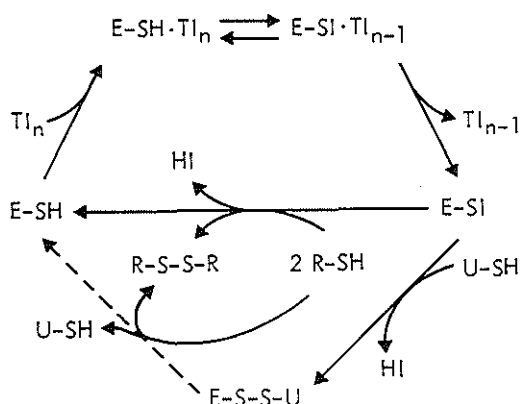deiodinase-thiouracil disulfide (E-S-S-U; see Fig. 3.9.).

Fig. 3.9. Pathway of enzymatic deiodination of iodothyronines (TI_n); the
        role of cofactor (R-SH) and inhibition by thiouracil (U-SH).

Competitive attenuation of the inhibitory effects by TU with methimazole
and thiourea suggests that all these compounds compete with cofactor for the
E-SI intermediate. The small effect by methimazole and thiourea on deiodin-
ation compared with that on iodination points to a different structural
requirement for inhibitors of these processes. The findings suggest that
methimazole (M-SH) also forms a mixed disulfide with the enzyme on reaction
with the SI group (E-SI + M-SH → E-S-S-M + HI). This disulfide would be rapid-
ly reduced by cofactor (E-S-S-M + 2 R-SH → E-SH + R-S-S-R + M-SH), whereas

the enzyme-thiouracil disulfide apparently is not. Amelioration of the inhibit-
ion by TU with methimazole may also be the result of a thiol-disulfide exchange
(E-S-S-U + M-SH → E-S-S-M + U-SH) followed by rapid reduction of the enzyme-
methimazole disulfide. It was found that methimazole is not a cofactor of
deiodinase catalysed reactions (unpublished results), excluding reactions such
as E-SI + 2 M-SH → E-SH + M-S-S-M + HI and, therefore, E-S-S-M + M-SH → E-SH +
M-S-S-M.

Finally, sulfite has similar effects on the kinetics of enzymatic deiodin-
ation, suggesting that it also is a dead-end inhibitor (Dixon and Webb, 1979),
acting on the E-SI intermediate. This would yield a thiosulfate: E-SI + HSO_3 →
E-S-SO_3^- + HI (Parker and Kharash, 1959; Visser, 1980a). The additive rather
than competitive action of methimazole in the presence of sulfite may indicate
that HSO_3^- also reacts with the enzyme-thioureylene disulfide (E-S-S-U or
E-S-S-M + HSO_3^- → E-S-SO_3^- + U-SH or M-SH; Parker and Kharash, 1959; Visser,
1980a).

Although the hypothesis concerning the involvement of an E-SI complex in
the deiodination of iodothyronines is attractive, the actual formation of such
intermediate is far from established. Due to the extreme lability of such
groups it would appear an almost impossible task to proof the validity of the
model. Experiments, however, may be designed, by which circumstantial evidence
for the proposed mechanism may be obtained. Apparently covalent binding of
radioactive TU to the 5'-deiodinase, which only takes place in the presence
of substrate (and, therefore, only after the formation of the E-SI intermediate)
is one such piece of evidence (Visser and Van Overmeeren, 1979; Leonard and
Rosenberg, 1980). Also the irreversible inactivation of the enzyme by TU which
only occurs in the presence of substrate (Leonard and Rosenberg, 1978b, 1980;
Visser and Van Overmeeren, 1980b) are in line with the hypothesis. In these
inactive complexes, TU is probably linked to the enzyme via a S-S bond, as
suggested by the finding that DTT not only prevents their formation but also
regenerates free enzyme and thiouracil once these complexes have been formed.

On the assumption that enzymatic deiodination of iodothyronines follows
the pathway depicted in Fig. 3.9., there have been speculations on the molec-
ular mechanism of these reactions (Visser, 1979). Deiodination of the phenolic
ring and that of the tyrosyl ring have several properties in common, i.e.
stimulation by thiols and uncompetitive inhibition by TU (e.g. Chopra et al,
1978c). This suggests a common underlying mechanism for both reactions, and
thereby seems to dispose of one of the possibilities, which have been consider-
ed. This reaction mechanism was based on the observations by Hartmann and co-

workers (Hartmann et al, 1971a,b; see also Friedman, 1973) of the non-enzymatic
deiodination of DIT by cysteine. Although formation of an E-SI complex by this
mechanism is entirely feasible, this possibility was rejected since it requires
the presence of a phenolic hydroxyl group in the ortho position to the iodine
substituent. Therefore, deiodination of the tyrosyl ring is not possible (Vis-
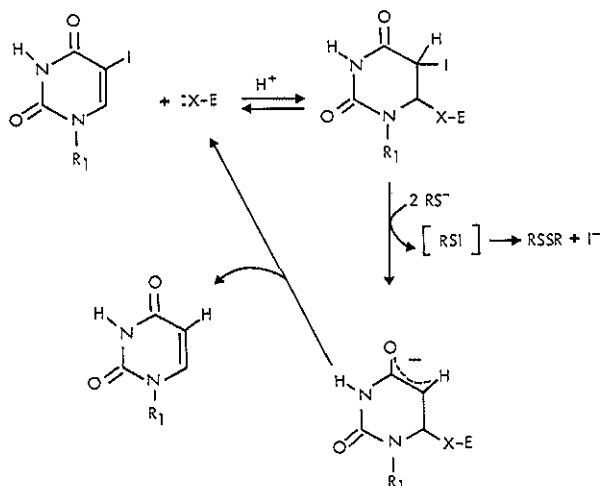ser, 1979).

Fig. 3.10. Mechanism of deiodination of 5-iodo-2'-deoxyuridylate by thymidylate
       synthetase (according to Wataya and Santi, 1975).

A second possibility was considered more likely, which is related to the
mechanism of action of thymidylate synthetase in the reductive deiodination of
5-iodo-2'-deoxyuridylate (see Fig. 3.10.) as elucidated by Santi and coworkers
(Wataya and Santi, 1975; Pogolotti and Santi, 1977; Garrett et al, 1979; see
also Sander, 1977; Chikuma et al, 1978). Applying this model to the reductive
deiodination of iodothyronines (see Fig. 3.11.) implicates a primary attack of
an enzyme nucleophile (X) at the 6' (or the equivalent 2') position. This
results in the formation of a covalent 5',6'-dihydrosubstrate-enzyme complex.
Elimination of I^+ in a concerted mechanism with the aid of an enzyme-thiolate
anion yields the E-SI complex and monodeiodinated substrate.

Any proposed mechanism of action of iodothyronine deiodinase is based
mainly on speculation. The above model, however, suffices as a working hypo-
thesis as it can be verified by experimental testing. It is an attractive model
since both phenolic and tyrosyl ring deiodination may occur via such a mechan-
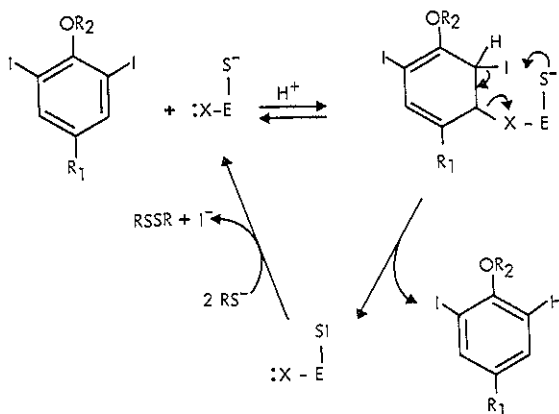ism. Moreover, it may explain why rT_3 and $3',5'-T_2$ are better substrates for

Fig. 3.11. Possible mechanism of enzymatic 5- and 5'-deiodination.

the 5'-deiodinase than T_4 (Visser et al, 1979a; Visser and Van Overmeeren, 1980a). Since 5'-deiodination is supposed to involve the reaction of an enzyme-nucleophile with $C^{6'}$ (or $C^{2'}$), bulky iodine substituents on C^3 and C^5 may interfere with the formation of a proper contact between enzyme and substrate. Relief from steric hindrance by deletion of one or both of these iodine atoms may, therefore, account for the preferred reactions with rT_3 and $3',5'-T_2$. To test the validity of the model, substrate analogues could be prepared - in analogy with thymidylate synthetase-catalysed reactions (Matsuda et al, 1978; Mertes et al, 1978; Brouillette et al, 1979) - which would arrest the catalytic cycle at the stage of the 5',6'-dihydrosubstrate-enzyme complex. The most obvious compounds to test would be nitrothyronine derivatives. Finally, to stress the speculative nature of the above discussion it is mentioned that even the stoichiometry of enzymatic iodothyronine deiodination with respect to cofactor oxidation has not been settled.

The here described mechanism for iodothyronine-deiodinating enzymes is clearly different from that for iodotyrosine deiodinase. The latter is also a microsomal enzyme occurring in the thyroid as well as in the liver. It has been characterized as a flavoprotein, containing FMN, and reductive equivalents may be supplied either by NADPH through an electron carrier with its specific reductase, or directly by dithionite (Rosenberg and Goswami, 1979; Goswami and Rosenberg, 1979).

Thyroid hormone deiodination is not mediated by tyrosine hydroxylase (Rokos and Scheiffele, 1979), nor by thymidylate synthetase (Visser et al, 1978c).

Irrespective of the exact molecular mechanism of inhibition by TU, it is evident that coincubation with substrate is a prerequisite for inactivation of iodothyronine deiodinase. This offers the possibility to assess more directly which iodothyronines are substrate for a common enzyme. It was found that pre-incubation of rat liver microsomes with TU and low concentrations of T_4, rT_3 or $3',5'-T_2$ resulted in the persistent and concomitant diminution of rT_3 and $3',5'-T_2$ 5'-deiodinase activity (Visser and Van Overmeeren, 1980b). Preincubations with TU only or iodothyronines only or with TU in the presence of T_0 or DIT were without effect. Lower concentrations of rT_3 and $3',5'-T_2$ than of T_4 were needed to achieve a similar degree of inactivation. As rT_3 and $3',5'-T_2$ are also more readily deiodinated at the 5' position than T_4, these findings lend support for the supposition that deiodination of substrate and, thus, iodo-enzyme complex formation preceeds inactivation by TU. The results demonstrate that indeed T_4, rT_3 and $3',5'-T_2$ are substrates for a single 5'-deiodinase. Surprisingly, it was found that high concentrations of $3,5-T_2$ also assisted in the production of inactive enzyme-TU complexes. This indicates the possibility of one enzyme mediating the sequential breakdown of T_4 by both 5- and 5'-deiodination. The alternative explanation still is the two enzyme-concept implicating preference rather than selectivity of the 5- and 5'-deiodinase with respect to the position of the iodine atoms in the substrate (Visser and Van Overmeeren, 1980b). Up to now it has not been possible to separate 5- from 5'-deiodinase activity in detergent extracts of liver microsomes (Fekkes et al, 1980).

3.9. Conclusions

With respect to the metabolism of iodoamino acids, three types of deiodinative reactions may be distinguished: i) oxidative breakdown of iodothyronines with release of iodide, ii) enzymatic, reductive deiodination of iodothyronines, and iii) enzymatic, reductive deiodination of iodotyrosines. In the presence of tissue components both enzymatic and non-enzymatic pathways are involved with the oxidative breakdown of T_4. Similar degradation may, however, also occur in the absence of tissue preparations, provided that conditions allow for the generation of free radical species. The initial step is probably the formation of a phenoxy radical. Among the products are iodide, derived from the phenolic ring, and DIT. Iodination of protein, if present, has frequently been observed. The physiological significance of this reaction is considered minimal, because of mechanisms within the cell, which prevent the creation of the hazardous conditions necessary for this type of deiodination. Nevertheless,

quite extensive metabolism of T_4 by such a mechanism may take place in vivo
in phagocytosing leucocytes.

The oxidative degradation of T_4 has for a long time interfered with the
detection of the specific conversion into T_3 and rT_3 and, subsequently, lower
substituted iodothyronines in vitro. However, the introduction in recent years
of reliable techniques to measure the specific metabolites has made it possible
to study this second type of deiodination. Evidence has been presented that
these reactions are enzymatic in nature. The enzymes are located in the endo-
plasmic reticulum of liver and, possibly, in the plasma membrane fraction of
kidney homogenate. Mercapto compounds are required for deiodinase activity.
Thiouracils are potent inhibitors of these reactions. Based on the kinetics of
stimulation by thiols and inhibition by TU, a ping-pong mechanism has been
indicated. According to the proposed model, the reductive deiodination of iodo-
thyronines is envisaged as a transfer of an iodinium ion from the substrate to
a receptive group on the enzyme, followed by the reduction of this iodo-enzyme
complex by thiol groups of the cofactor. The specific reaction of thioureylenes
with sulfenyl iodides point to the formation of such an enzyme derivative:
TI_n (substrate) + E-SH (enzyme) → TI_{n-1} (first product) + E-SI (intermediate)
E-SI + 2R-SH (cofactor) → E-SH + R-S-S-R + HI (second product).

It has been shown that T_4, rT_3 and $3',5'-T_2$ are substrates for a common
enzyme catalyzing the deiodination of the phenolic ring. However, $3,5-T_2$ also
appeared to be deiodinated by this enzyme, though with less efficiency. This
may indicate either that all possible deiodinations are mediated by a single
enzyme or that two enzymes are required (iodothyronine 5- and 5'-deiodinase)
which do not exhibit full specificity as far as the positions of the iodine
substituents in the substrate are concerned. Deiodination of the phenolic ring
(at low substrate concentrations) is optimal at slightly acidic pH, whereas
non-phenolic ring deiodination proceeds maximally in a slightly alkaline milieu.
As various manipulations affect deiodinase activity of isolated tissues in the
same way as observed in the intact organism, it is concluded that these in vitro
observations are representative for the physiological situation (see Table 3.3.).

The third type of reaction concerns the enzymatic deiodination of iodo-
tyrosines. This is catalysed by a flavoprotein from thyroid and liver micro-
somes, where reductive equivalents are supplied by NADPH via an as yet un-
identified electron carrier and its specific reductase.

Table 3.3. EFFECTS OF VARIOUS IN VIVO AND IN VITRO TREATMENTS ON THE CONVERSION OF T_4 INTO T_3 IN THE INTACT ORGANISM AND IN LIVER HOMOGENATES[a]

Treatment or condition	In vivo		In vitro
	Body T_4-T_3 conversion	Tissue T_4-T_3 conversion	Tissue T_4-T_3 conversion
Starvation	↓	↓	-
Carbohydrate feeding	↑	↑	-
Diabetes mellitus	↓	↓	-
PTU	↓	↓	↓
Methimazole	=	=	=
Dexamethasone	↓	↓	=
Propranolol	↓	-	=
X-ray contrast agents	↓	↓	↓
rT_3	↓	↓	↓
α-Methyltyrosine	=	-	=
Diphenylhydantoin	↑	↑	=

[a] ↑, increased; ↓, decreased; =, unaffected; -, not tested or not applicable.

4.   REGULATION OF THYROID HORMONE DEIODINATION

4.1. Introduction

Several factors must be considered to play a role in the regulation of peripheral thyroid hormone deiodination, i.e.: changes in i) substrate availability, ii) concentration of active enzymes, iii) concentration of cofactor, iv) concentration of naturally occurring inhibitors, and v) pH.

While the enzymatic mechanism of iodothyronine deiodination is still only partly understood and the basic question concerning the number of enzymes involved has as yet not been resolved, several attempts have been made to identify the cause of a change in T_4 metabolism. Most attention has been given to the effects of nutritional and thyroid status on deiodinase activity of tissue preparations.

4.2. Effects of nutritional and thyroid status

Several studies have demonstrated that the deiodinase activity of unsupplemented rat liver homogenates is decreased during fasting (Balsam et al, 1978b, 1979a; Harris et al, 1978b,c, 1979; Kaplan and Utiger, 1978a; Kaplan, 1979a; Kaplan et al, 1979; Chopra, 1980; Gavin et al, 1980a,b). In contrast, no effect of fasting was observed in rat kidney homogenate (Kaplan et al, 1979) and pituitary fragments (Cheron et al, 1979). In addition, "fasting" was found to have no effect on the 5'-deiodination of rT_3 in monkey hepatocarcinoma cells, whereas production of rT_3 from T_4 was even increased (Sorimachi and Robbins, 1978a). Production of T_3 from T_4 by perfused rat livers from fasted rats was found to be normal if related to the amount of T_4 taken up. The latter was found to be decreased (Jennings et al, 1979).

In rats, prolonged starvation is accompanied by decreased T_4 and T_3 levels in the plasma. Hypothyroidism has been shown to lead to diminished deiodinase activities of the tissues, whereas hyperthyroidism results in enhanced activities (Larson et al, 1955; Grussendorf and Hüfner, 1977; Balsam et al, 1978a, 1979b; Harris et al, 1978b, 1979; Kaplan and Utiger, 1978b; Kaplan, 1979b). A notable exception is the converting activity of the anterior pituitary (Cheron et al, 1979; Kaplan, 1980).

Several studies have indicated that the defect in rat liver 5'-deiodinase activity induced by a 2-days fast is related to changes in intracellular GSH concentrations. This was suggested from the findings that i) liver GSH content is decreased during fasting (Harris et al, 1979; Chopra, 1980) ii) there was no

difference between deiodinase activity of "fasted" and "fed" homogenates if this was measured after addition of DTT (Harris et al, 1979; Chopra, 1980), iii) the activity of the microsomal fraction tested with exogenous thiols was not affected (Balsam et al, 1979a), iv) the potency of liver cytosol to support deiodination by microsomes was diminished (Balsam et al, 1979a) and v) this diminished potency could not only be restored by the addition of GSH but also with NADPH (Balsam et al, 1979a). Noteworthy are also the findings by Kaplan et al (1979) who could not find an effect of fasting on deiodinase activity of rat kidney homogenates. In contrast to liver, kidney GSH content was found to be unaffected.

General agreement exists that there is a direct influence of thyroid status on enzyme function rather than on GSH concentrations (Balsam et al, 1979b; Harris et al, 1979; Kaplan, 1979b). Studies have been conducted to exclude that fasting-induced changes in peripheral thyroid-hormone metabolism are secondary to a decrease in thyroid function. In these studies, the effects of thyroid hormone administration during fasting were investigated. It was found that the decrease in tissue deiodinase activity by short-term (2 days) starvation was not reverted by hormone substitution (Harris et al, 1978b, 1979; Chopra, 1980). These results indicate different underlying mechanisms for the decreased 5'-deiodinase activity of liver in early fasting and hypothyroidism. However, during prolonged starvation an impairment of thyroid function appears to play an important role (Chopra, 1980). This may explain the disparity observed between tissue non-protein-SH content and deiodinase activity after 3 or 4 days of fasting (Kaplan, 1979; Chopra, 1980; Gavin et al, 1980a).

Thus, in the early phase of starvation, decreased peripheral production rates of T_3 may be accounted for by a decline in the concentration of cofactor. In a later phase, tissue converting activity may be further impaired by a decrease in the number of enzyme units due to ensuing hypothyroidism. This dual mechanism is also apparent from the finding by Burger et al (1980), that refeeding rats following 6 days of starvation – in contrast to shorter periods – did not induce an acute rise of serum T_3. As fasting has no profound effects on thyroid function in man, this prolonged defect of peripheral thyroid hormone metabolism may be dissimilar in rats.

Even in short-term fasting, reduction of intracellular GSH concentrations cannot be the sole cause for the decreased deiodinase activity of rat liver. Although many studies have emphasized the importance of carbohydrate intake for optimal peripheral T_3 production, protein depletion has been recognized as the predominant factor in the lowering of liver GSH content by fasting (Leaf and Neuberger, 1947; Edward and Westerfeld, 1952; Barford and Eden, 1956).

More specifically, GSH levels are related to the intake of cysteine, cystine and methionine (Tateishi et al, 1977). This contradistinction is substantiated by the work of Gavin et al (1980b), who found that GSH concentrations were highest in rats fed a protein-rich diet, whereas deiodination was most effective in homogenates from rats fed glucose only. Moreover, fortifying the glucose diet with cysteine resulted in a rise of GSH levels, without a discernable effect on deiodination.

It may be postulated that deiodinase activity is determined by the glutathione sulfhydryl-disulfide ratio rather than by GSH levels per se (Fig. 4.1.). This is strengthened by the observation of an inhibitory effect of GSSG on tissue T_4-T_3 converting activity (Chopra, 1978), which may be due to the formation of a mixed disulfide (E-S-SG) with an essential SH group. The activity of other enzymes (e.g. fructose-1,6-diphosphatase and glycogen synthetase) is also regulated by enzyme-glutathione mixed disulfide formation (Isaacs and Binkley, 1977a,b).
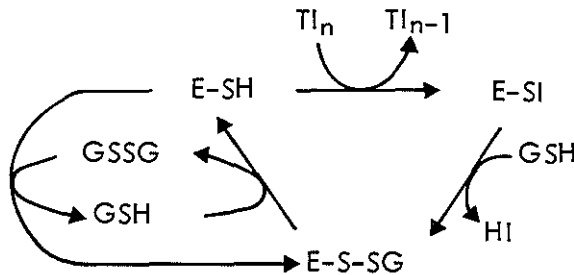

Fig. 4.1. Possible regulation of thyroid hormone deiodination by GSH/GSSG ratio

It has previously been suggested (Visser, 1978; Balsam et al, 1979a) that fasting-induced changes may be explained by a diminished production of NADPH in the hexose monophosphate shunt due to a lack of substrate (glucose). Since NADPH is a cofactor in the reduction of glutathione by glutathione reductase this will lead to a decrease in GSH levels (or the glutathione sulfhydryl-disulfide ratio) and, consequently, to a decrease in deiodinase activity (Fig. 4.2.). However, fasting does not induce a fall in liver NADPH concentrations (Greenbaum et al, 1971; Isaacs and Binkley, 1977b).

The following sequence of events may seem more likely. In fasting, glucagon secretion is increased which stimulates adipose tissue to mobilize fatty acids and the liver to oxidize these fatty acids. This results in increased productions of H_2O_2 which is metabolised via glutathione peroxidase at the expense of GSH. The GSSG produced will then react with protein sulfhydryls to form mixed
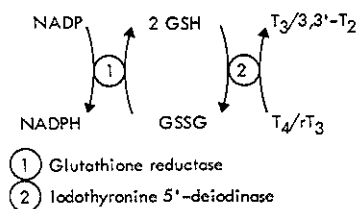
Fig. 4.2. The role of NADPH and GSH in thyroid hormone deiodination

disulfides(Isaacs and Binkley, 1977b).

In conclusion, it is suggested that oxidation of glutathione is an important factor in the decrease of tissue 5'-deiodinase activity as the underlying mechanism for a defect in the peripheral metabolism of T_4 during dietary restrictions and perhaps also in systemic illness. For this it is necessary to assume that the deiodination itself is the rate limiting step in the peripheral conversion of thyroid hormone. An additional problem here is that not only 5'-deiodinase activity but also the 5-deiodinase is dependent on the availability of sulfhydryl groups. Nevertheless, in the intact organism, fasting is associated with a selective decrease of 5'-deiodinase catalysed reactions, leaving the 5-deiodination unaffected.

The analogy between starvation and the effect of PTU in vivo is apparent. Administration of PTU evokes the manifestations of a selective decrease in 5'-deiodinase activity, i.e. decreased serum T_3 and increased serum rT_3 concentrations. Nevertheless, derivatives of TU have been shown to inhibit both types of deiodination in vitro. It has been demonstrated that addition of TU and a decrease in the concentration of thiol compounds have identical effects on the kinetics of enzymatic deiodination. Both manipulations interfere with the reduction of the E-SI intermediate (see previous chapter). It may of course be argued that the 5'-deiodinase is more sensitive to these manipulations than the 5-deiodinase (if at all these are separate enzyme entities). Since there is not sufficient experimental evidence to support this assumption, this must remain purely speculative.

Additional regulatory mechanisms for fasting-induced changes in the peripheral metabolism of thyroid hormone have been suggested. Jennings et al (1979) investigated conversion of T_4 into T_3 with the perfused rat liver. They found that livers from fasted rats took up less T_4 from the medium and produced less T_3 as compared with livers from normally fed controls. If the production of T_3

was related to the amount of T_4 taken up by the liver, no abnormality could be observed. These authors concluded that the deiodination itself is not the rate-limiting step in the conversion of T_4 into T_3 but rather the transport of the substrate from the vascular to the intracellular compartment. A complicating factor in these studies was, however, the presence of glucose in the perfusion medium. Nevertheless, these findings suggest that variations in thyroid hormone transport through the plasma membrane play a part in the regulation of the peripheral T_4 metabolism. This does not appear unlikely as it has been found that this transport is an active process, being dependent on intracellular ATP concentrations (Krenning et al, 1978, 1979, 1980). Cytoplasmic ATP is strongly diminished by fasting (Soboll et al, 1978).

Reduced uptake of substrate can not be the sole explanation for the reduced conversion of T_4 into T_3 since in that case one would expect a concomitant reduction in the conversion of T_4 into rT_3, which also takes place intracellularly. Unless most rT_3 is produced outside the liver, for which there is no experimental evidence (see Fig. 4.3.). The hypothesis that changes in intracellular pH are also involved in the modulation of the deiodination processes has been put forward by the group of Hesch. In their view, changes in pH could have an effect either on the availability of substrate by influencing the binding to intracellular proteins (Höffken et al, 1977) or on the activity of the deiodinating enzymes (Hesch et al, 1980). These authors suggest that in the latter situation a change in intracellular pH will result in a diminished degradation (by 5'-deiodination) of rT_3. The accumulation of this inactive metabolite will in turn lead (by competitive inhibition) to a decreased conversion of T_4 into T_3.

4.3. General conclusions

It is generally conceived that T_3 is the most active form of thyroid hormone. Besides the small amount secreted by the thyroid most of circulating T_3 is derived from peripheral 5'-deiodination of T_4. An alternative product, rT_3, is obtained by 5-deiodination of T_4. The biological potency of rT_3 is negligible and so is probably the intrinsic activity of T_4. The nature of thyroid hormone synthesis suggests that it is directed to keeping the level of inactive precursor at a constant level. The amount of active hormone produced is then regulated at the level of peripheral tissues, i.e. at the site of the target organs. Several clinical observations tend to suggest that indeed the amount of T_3 produced is adjusted to the need which exists in specific circumstances. It appears that this is mainly achieved by the regulation of 5'-deiodinase activity. In vitro studies of the deiodination of iodothyronines, mainly
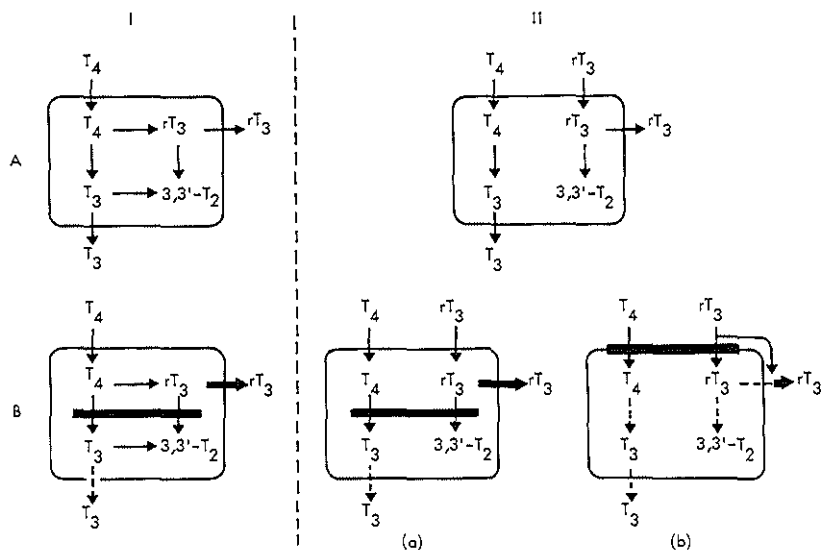
Fig. 4.3. Possible mechanisms for normal (A) or decreased (B) production of T_3 (by 5'-deiodination from T_4) and degradation of rT_3 (by 5'-deiodination into 3,3'-T_2).
I : 5- and 5'-deiodination take place in the same cell (tissue). Observations can only be explained by a selective decrease in 5'-deiodinase activity. A general decrease in deiodinase activity or a decrease in substrate uptake will also result in a diminished rT_3 production.
II: 5- and 5'-deiodination take place in different cells (tissues). Observations can be explained by a defect in the 5'-deiodinating cell (tissue): decrease in deiodinase activity (a) or in substrate uptake (b).

involving rat liver preparations, have therefore been performed to elucidate the molecular mechanism of this regulation.

These in vitro studies have indicated that all possible deiodinations are enzymatic in nature, where the enzymes are located in the microsomal fraction (endoplasmic reticulum). The activity of these enzymes depends on the availability of thiols in the cytoplasmic fraction, and GSH is probably the endogenous cofactor. Circumstantial evidence has been presented that the entire sequential deiodination is catalysed by two enzymes, i.e. iodothyronine 5- and 5'-deiodinase. It has, however, also been demonstrated that if this proves to be correct, the enzymes do not display full specificity with regard to the position of the iodine substituents in the substrate. It may, therefore, be quite possible that the 5'-deiodinase also mediates 5-deiodinations, though with low efficiency, and vice versa. Based on the effects of TU and variations in cofactor concentration on the kinetics of the 5'-deiodination, a model has

been developed in which the reaction is envisaged as consisting of two half-reactions. In the first, there is a transfer of an iodinium ion from the substrate to a sulfhydryl group of the enzyme. In the second half-reaction this intermediate iodo-enzyme complex is reduced by cofactor to free enzyme (ping-pong mechanism).

Armed with this rather poor knowledge of the deiodinating enzymes and the reactions they catalyse, attempts have been made to explain the observations of an altered metabolism in experimental conditions, notably during starvation. Most investigations have centered around the role of glutathione in the determination of deiodinase activity. They have revealed an association between the oxidation state of glutathione and the rate of 5'-deiodination during fasting. Several observations remain unexplained, however, which has led to the proposal of additional regulatory mechanisms. These involve changes in cellular uptake of T_4 and changes in intracellular pH. Neither of these in itself can explain the observed alteration in thyroid hormone metabolism. This illustrates the complex nature of this matter and it seems pertinent that basic knowledge of the enzymes is increased, before definite answers on questions concerning this regulation can be given. A better understanding of the reactions will depend on the availability of pure enzyme preparations.

REFERENCES

Abrams, G.M. and P.R. Larsen (1973). J. Clin. Invest. 52: 2522-2531.

Abuid, J. and P.R. Larsen (1974). J. Clin. Invest. 54: 201-208.

Adlkofer, E., D.B. Ramsden, M.C. Wusteman, D.E. Pegg and R. Hoffenberg (1977). Hormone Metab. Res. 9: 400-403.

Afrasiabi, M.A., N.B. Vaziri, G. Gwinup, D.M. Mays, C.H. Barton, R.L. Ness and N.J. Valenta (1979). Ann. Intern. Med. 90: 335-338.

Albright, E.C. and F.C. Larson (1959). J. Clin. Invest. 38: 1899-1903.

Albright, E.C., F.C. Larson and R.H. Trust (1954). Proc. Soc. Exp. Biol. Med. 86: 137-140.

Anderberg, B., B. Kågedal, O.R. Nilsson, S. Smeds, L. Tegler and J. Gillqvist (1979). Acta Chir. Scand. 145: 297-303.

Andik, I., L. Balogh and Sz. Donhoffer (1949). Experientia 6: 249-250.

Astwood, E.B. (1943). J. Pharmacol. Exp. Ther. 78: 79-89.

Astwood, E.B., A. Bissell and A.M. Hughes (1945). Endocrinology 37: 456-481.

Auf dem Brinke, D., R-D. Hesch and J. Köhrle (1979). Biochem. J. 180: 273-279.

Auf dem Brinke, D., J. Köhrle, R. Ködding and R-D. Hesch (1980). J. Endocrinol. Invest. 3: 73-76.

Azizi, F. (1978). Metabolism 27: 935-942.

Azizi, F., A.G. Vagenakis, J.E. Busch, L.E. Braverman (1974). Metabolism 23: 525-529.

Balsam, A. and S.H. Ingbar (1978). Clin. Res. 26: 247A.

Balsam, A., F. Sexton and S.H. Ingbar (1978a). Endocrinology 103: 1759-1767.

Balsam, A., S.H. Ingbar and F. Sexton (1978b). J. Clin. Invest. 62: 415-424.

Balsam, A., S.H. Ingbar and F. Sexton (1979a). J. Clin. Invest. 63: 1145-1156.

Balsam, A., F. Sexton and S.H. Ingbar (1979b). Endocrinology 105: 1115-1121.

Barford, H. and E. Eden (1956). Aust. J. Exp. Biol. 34: 269-276.

Barker, S.B., C.E. Kiely and H.J. Lipner (1949). Endocrinology 45: 624-627.

Barker, S.B., H.B. Dirks, W.R. Garlick and H.M. Klitgaard (1951). Proc. Soc. Exp. Biol. Med. 78: 840-845.

Bastomsky, C.H. and K-T. Lin (1979). Biochem. Pharmacol. 28: 443-444.

Becker, D.V. and J.F. Prudden (1959). Endocrinology 64: 136-148.

Benua, R.S., S. Kumaoka, R.D. Leeper and R.W. Rawson (1959). J. Clin.Endocrinol. Metab. 19: 1344-1346.

Bernal, J. and F. Escobar del Rey (1974). Acta Endocrinol. 77: 276-281.

Bernal, J. and F. Escobar del Rey (1975a). Hormone Metab. Res. 7: 222-227.

Bernal, J. and F. Escobar del Rey (1975b). Acta Endocrinol. 78: 481-492.

Birkhäuser, M., R. Busset, Th. Burer and A. Burger (1977). Lancet II: 53-56.

Boonnamsiri, V., J.C. Kermode and B.D. Thompson (1979). J. Endocrinol. 82: 235-241.

Brandt, M.R., L. Skovsted, H. Kehlet and J.M. Hansen (1976). Lancet II: 1333-1336.

Braverman, L.E. and S.H. Ingbar (1962). Endocrinology 71: 701-712.

Braverman, L.E. and A.G. Vagenakis (1979). Clin. Endocrinol. Metab. 8: 621-639.

Braverman, L.E., S.H. Ingbar and K. Sterling (1970). J. Clin. Invest. 49: 855-864.

Braverman, L.E., A. Vagenakis, P. Downs, A.E. Foster, K. Sterling and S.H. Ingbar (1973). J. Clin. Invest. 52: 1010-1017.

Bray, G.A. (1977). Pharmacol. Ther. (C) 2: 205-214.

Bray, G.A. and S. Hildreth (1967). Endocrinology 81: 1018-1020.

Bray, G.A., D.A. Fisher and I.J. Chopra (1976). Lancet I: 1206-1208.

Britton, K.E., S.M. Ellis, J.M. Miralles, V. Quinn, A.C.D. Cayley, B.L. Brown and R.P. Ekins (1975). Lancet II: 141-142.

Brouillette, C.B., C.T-C. Chang and M.P. Mertes (1979). Biochem. Biophys. Res. Commun. 87: 613-618.

Brown, M.R. and G.A. Hedge (1973). Endocrinology 92: 1305-1311.

Brown, M.R. and G.A. Hedge (1974). Amer. J. Physiol. 227: 289-294.

Burger, A. and C. Alliod (1977). Ann. Endocrinol. 38: 52A.

Burger, A., D. Dinichert, P. Nicod, M. Jenny, T. Lemarchand-Béraud and M.B. Vallotton (1976). J. Clin. Invest. 58: 255-259.

Burger, A.G., M. Berger, K. Wimpfheimer and E. Danforth (1980). Acta Endocrinol. 93: 322-331.

Bürgi, H., C. Wimpfheimer, A. Burger, W. Zaunbauer, H. Rösler, T. Lemarchand-Béraud (1976). J. Clin. Endocrinol. Metab. 43: 1203-1210.

Burman, K.D., Y. Lukes, F.D. Wright and L. Wartofsky (1977). Endocrinology 101: 1331-1334.

Burman, K.D., F.D. Wright, R.C. Smallridge, B.J. Green, L.P. Georges and L. Wartofsky (1978). J. Clin. Endocrinol. Metab. 47: 1059-1064.

Burman, K.D., R.C. Dimond, G.S. Harvey, J.T. O'Brian, L.P. Georges, J. Bruton, F.D. Wright and L. Wartofsky (1979a). Metabolism 28: 291-299.

Burman, K.D., L. Wartofsky, R.E. Dinterman, P. Kesler and R.W. Wannemacher (1979b). Metabolism 28: 805-813.

Burman, K.D., R.C. Smallridge, R. Osburne, R.C. Dimond, N.E. Whorton, P. Kesler and L. Wartofsky (1980). Metabolism 29: 46-52.

Burr, W.A., E.G. Black, R.S. Griffiths, R. Hoffenberg, H. Meinhold and K.W. Wenzel (1975). Lancet II: 1277-1279.

Burr, W.A., R.S. Griffiths, D.B. Ramsden and E.G. Black (1976). Lancet II: 58-61.

Carlson, H.E., E.J. Drenick, I.J. Chopra and J.M. Hershman (1977). J. Clin. Endocrinol. Metab. 45: 707-713.

Cavalieri, R.R., M. Steinberg and G.L. Searle (1971). J. Clin. Endocrinol.Metab. 33: 624-629.

Cavalieri, R.R., L.C. Sung and C.E. Becker (1973). J. Clin. Endocrinol. Metab. 37: 308-316.

Cavalieri, R.R., L.A. Gavin and F. Bui (1977). Biochem. Biophys. Res. Commun. 79: 897-902.

Cavalieri, R.R., L.A. Gavin, A. Wallace, M.E. Hammond and K. Cruse (1979). Metabolism 28: 1161-1165.

Cheron, R.G., M.M. Kaplan and P.R. Larsen (1979). J. Clin. Invest. 64: 1402-1414.

Chikuma, T., K. Negishi and H. Hayatsu (1978). Chem. Pharm. Bull. 26: 1746-1752.

Chiraseveenuprapund, P., U. Buergi, A. Goswami and I.N. Rosenberg (1978). Endocrinology 102: 612-622.

Chopra, I.J. (1974). J. Clin. Invest. 54: 583-592.

Chopra, I.J. (1976). J. Clin. Invest. 58: 32-40.

Chopra, I.J. (1977). Endocrinology 101: 453-463.

Chopra, I.J. (1978). Science 199: 904-906.

Chopra, I.J. (1980). Metabolism 29: 161-167.

Chopra, I.J., D.H. Solomon, U. Chopra, R.T. Young and G.N. Chua Teco (1974). J. Clin. Endocrinol. Metab. 39: 501-511.

Chopra, I.J., D.E. Williams, J. Orgiazzi and D.H. Solomon (1975). J. Clin. Endocrinol. Metab. 41: 911-920.

Chopra, I.J., D.H. Solomon, U. Chopra, S.Y. Wu, D.A. Fisher and Y. Nakamura (1978a). Rec. Progr. Horm. Res. 34: 521-567.

Chopra, I.J., F. Geola, D.H. Solomon and R.M.B. Maciel (1978b). J. Clin. Endocrinol. Metab. 47: 1198-1207.

Chopra, I.J., S.Y. Wu, Y. Nakamura and D.H. Solomon (1978c). Endocrinology 102: 1099-1105.

Chopra, I.J., D.H. Solomon, G.W. Hepner and A.A. Morgenstein (1979a). Ann.Intern. Med. 90: 905-912.

Chopra, I.J., G.N. Chua Teco, A. Nguyen and D.H. Solomon (1979b). J. Clin. Endocrinol. Metab. 49: 63-69.

Chopra, I.J., D.H. Solomon and S.Y. Wu (1979c). J. Endocrinol. Invest. 2: 462-463.

Chow, C.K. (1979). Amer. J. Clin. Nutr. 32: 1066-1081.

Ciaraldi, T. and G.V. Marinetti (1977). Biochem. Biophys. Res. Commun. 74: 984-991.

Ciaraldi, T.P. and G.V. Marinetti (1978). Biochim. Biophys. Acta 541: 334-346.

Coiro, V., A. Harris, H.M. Goodman, A. Vagenakis and L. Braverman (1980). Endocrinology 106: 68-75.

Costa, A. (1979). J. Endocrinol. Invest. 2: 461-462.

Crantz, F.R. and P.R. Larsen (1980). J. Clin. Invest. 65: 935-938.

Croxson, M.S. and H.K. Ibbertson (1977). J. Clin. Endocrinol. Metab. 44: 167-174.

Croxson, M.S., T.D. Hall and J.T. Nicoloff (1977a). J. Clin. Endocrinol. Metab. 45: 623-630.

Croxson, M.S., T.D. Hall, O.A. Kletzky, J.E. Jaramillo and J.T. Nicoloff (1977b). J. Clin. Endocrinol. Metab. 45: 560-568.

Cruchaud, S., C. Mahaim, A. Vannotti and J. Deckelmann (1955). Lancet II: 906-907.

76

Cunningham, L.W. (1964). Biochemistry 3: 1629-1634.

Danforth, E., E.S. Horton, M. O'Connell, E.A.H. Sims, A.G. Burger, S.H. Ingbar, L. Braverman and A.G. Vagenakis (1979). J. Clin. Invest. 64: 1336-1347.

Davidson, B., M. Soodak, J.T. Neary, H.V. Strout, J.D. Kieffer, H. Mover and F. Maloof (1978). Endocrinology 103: 871-882.

Davidson, B., M. Soodak, H.V. Strout, J.T. Neary, C. Nakamura and F. Maloof (1979). Endocrinology 104: 919-924.

Davidson, M.B. and I.J. Chopra (1979). J. Clin. Endocrinol. Metab. 48: 577-581.

Davis, P.J. (1979). Clin. Endocrinol. Metab. 8: 603-619.

Dawber, N.A., V.A. Galton and S.H. Ingbar (1971). Endocrinology 88: 144-148.

DeGroot, L.J. and K. Hoye (1976). J. Clin. Endocrinol. Metab. 42: 976-978.

DeGroot, L.J. and H. Niepomniszcze (1977). Metabolism 26: 665-718.

DeGroot, L.J. and P.A. Rue (1979). J. Clin. Endocrinol. Metab. 49: 538-542.

DeGroot, L.J., A.H. Coleoni, P.A. Rue, H. Seo, E. Martino and S. Refetoff (1977). Biochem. Biophys. Res. Commun. 79: 173-178.

DeRubertis, F.R. (1974). J. Lab. Clin. Med. 81: 902-910.

DeRubertis, F.R. and K.A. Woeber (1973). J. Clin. Invest. 52: 78-87.

Dixon, M. and E.C. Webb (1980). "Enzymes", Longman, London.

Docter, R., T.J. Visser, J.T. Stinis, N.L. van den Hout-Goemaat and G. Hennemann (1976). Acta Endocrinol. 81: 82-95.

Dowling, J.T., D. Razevska and C.J. Goodner (1964). Endocrinology 75: 157-166.

Dratman, M.B. (1974). J. Theor. Biol. 46: 255-270.

Dratman, M.B., F.L. Crutchfield, J. Axelrod, R.W. Colburn and N. Thoa (1976a). Proc. Nat. Acad. Sci. US 73: 941-944.

Dratman, M.B., F.L. Crutchfield, E. Marsh, J. Axelrod and F.H. Sterling (1976b). In: "Thyroid Research". Eds. J. Robbins and L.E. Braverman. Excerpta Medica, Amsterdam, 248-250.

Dratman, M.B. and F.L. Crutchfield (1978). Amer. J. Physiol. 235: E638-E647.

Duick, D.S. and H.W. Wahner (1979). Arch. Intern. Med. 139: 767-772.

Duick, D.S., D.W. Warren, J.T. Nicoloff, C.L. Otis and M.S. Croxson (1974). J. Clin. Endocrinol. Metab. 39: 1151-1154.

Dussault, J.H. (1974). Can. Med. Ass. J. 7: 1195-1197.

Dvorak, J.C., K. Engelman and R.D. Utiger (1978). J. Clin. Endocrinol. Metab. 47: 442-444.

Edelhoch, H., G. Irace, M.L. Johnson, J.L. Michot and J. Nunez (1979). J. Biol. Chem. 254: 11822-11830.

Edwards, S. and W.W. Westerfeld (1952). Proc. Soc. Exp. Biol. Med. 79: 57-59.

Eisenstein, Z., S. Hagg, A.G. Vagenakis, S.L. Fang, B. Ransil, A. Burger, A. Balsam, L.E. Braverman and S.H. Ingbar (1978). J. Clin. Endocrinol. Metab. 47: 889-893.

Engler, D. and A.G. Burger (1978). 60th Annual Meeting of the Endocrine Society, abstract 82.

Engler, D., E.B. Donaldson, J.R. Stockigt and P. Taft (1978). J. Clin. Endocrinol. Metab. 46: 77-82.

Engler, D., U. Merkelbach and A.G. Burger (1979). Clin. Res. 27: 251A.

Epstein, Y., R. Udassin and J. Sack (1979). J. Clin. Endocrinol. Metab. 49: 677-678.

Escobar del Rey, F. and G. Morreale de Escobar (1962). Acta Endocrinol. 40: 1-15.

Escobar del Rey, F., G. Morreale de Escobar, M.D. García, J. Mouriz García (1962). Endocrinology 71: 859-869.

Etling, N. and S.B. Barker (1959). Endocrinology 95-100.

Faber, J., C. Kirkegaard, I.B. Lumholtz, K. Siersbaek-Nielsen and T. Friis (1979a). J. Clin. Endocrinol. Metab. 48: 611-617.

Faber, J., T. Friis, C. Kirkegaard, I.B. Lumholtz, J. Mølholm Hansen, K. Siersbaek-Nielsen, L. Skovsted and P. Thielade (1979b). Hormone Metab. Res. 11: 34-36.

Faglia, G., C. Ferrari, P. Beck-Peccoz, A. Spada, P. Travaglini and B. Ambrosi (1973). Hormone Metab. Res. 5: 289-292.

Feely, J., T.E. Isles, W.A. Ratcliffe and J. Crooks (1979). Clin. Endocrinol. 10: 531-538.

Fekkes, D., E. van Overmeeren-Kaptein, R. Docter, G. Hennemann and T.J. Visser (1979). Biochim. Biophys. Acta 587: 12-19.

Fekkes, D., E. van Overmeeren, G. Hennemann and T.J. Visser (1980). Biochim. Biophys. Acta 613: 41-51.

Fisher, D.A., J.H. Dussault, J. Sack and I.J. Chopra (1977). Rec. Progr. Horm. Res. 33: 59-116.

Fishman, N., Y.P. Huang, D.C. Tergis and R.S. Rivlin (1977). Endocrinology 100: 1055-1059.

Flock, E.V. and J.L. Bollman (1963). Biochem. J. 84: 621-626.

Flock, E.V. and J.L. Bollman (1964). Endocrinology 75: 721-732.

Flock, E.V. and C.A. Owen (1965). Amer. J. Physiol. 209: 1039-1045.

Flock, E.V., J.L. Bollman and J.H. Grindlay (1960a). Endocrinology 67: 419-429.

Flock, E.V., J.L. Bollman and J.H. Grindlay (1960b). Mayo Clin. Proc. 35: 75-81.

Flock, E.V., J.L. Bollman, J.H. Grindlay and G.H. Stobie (1961). Endocrinology 69: 626-637.

Flock, E.V., C. David, G.H.C. Stobie and C.A. Owen (1963). Endocrinology 73: 442-455.

Fregly, M.J., F.P. Field, M.J. Katovich and C.C. Barney (1979). Fed. Proc. 38: 2162-2169.

Friedman, M. (1973). "The Chemistry and Biochemistry of the Sulfhydryl group in Amino acids, Peptides and Proteins". Pergamon Press, Oxford, 170-172.

Frumess, R.D. and P.R. Larsen (1975). Metabolism 24: 547-554.

Furth, E.D., K. Rives and D.V. Becker (1966). J. Clin. Endocrinol. Metab. 26: 239-246.

Galeazzi, R.L. and A.G. Burger (1980). J. Clin. Endocrinol. Metab. 50: 148-151.

Galton, V.A. and S.H. Ingbar (1961). Endocrinology 69: 30-38.

Galton, V.A. and S.H. Ingbar (1962). Endocrinology 70: 210-220.

Galton, V.A. and S.H. Ingbar (1963). Endocrinology 73: 596-605.

Galton, V.A. and S.H. Ingbar (1964). Endocrinology 74: 627-634.

Galton, V.A. and S.H. Ingbar (1966). Endocrinology 78: 855-859.

Galton, V.A. and B.C. Nisula (1969). Endocrinology 85: 79-86.

Galton, V.A., S.H. Ingbar and S. von der Heyde (1965). Endocrinology 76: 479-485.

Gamstedt, A., G. Järnerot, B. Kågedal and B. Söderholm (1979). Acta Med. Scand. 205: 379-383.

Gardner, D.F., M.M. Kaplan, C.A. Stanley and R.D. Utiger (1979). N. Engl. J. Med. 300: 579-584.

Garrett, C., Y. Wataya and D.V. Santi (1979). Biochemistry 18: 2798-2804.

Gavin, L., J. Castle, F. McMahon, P. Martin, M. Hammond and R.R. Cavalieri (1977). J. Clin. Endocrinol. Metab. 44: 733-742.

Gavin, L.A., M. Hammond, J.N. Castle and R.R. Cavalieri (1978). J. Clin. Invest. 60: 1276-1285.

Gavin, L.A., F. Bui, F. McMahon and R. Cavalieri (1980a). J. Biol. Chem. 254: 49-54.

Gavin, L.A., F.A. McMahon and M. Moeller (1980b). J. Clin. Invest. 65: 943-946.

Geffner, D.L., M. Azukizawa and J.M. Hershman (1975). J. Clin. Invest. 55: 224-229.

Geola, F., I.J. Chopra, D.H. Solomon and R.M.B. Maciel (1979). J. Clin. Endocrinol. Metab. 48: 297-301.

Geola, F.L., I.J. Chopra and D.L. Geffner (1980). J. Clin. Endocrinol. Metab. 50: 336-340.

Gershengorn, M.C., E. Geras, B.E. Marcus-Samuels and M.J. Rebecchi (1979). Amer. J. Physiol. 237: E142-E146.

Gharib, H., R.J. Ryan, W.E. Mayberry and T. Hocket (1971). J. Clin. Endocrinol. Metab. 33: 509-516.

Glass, A.R., R. Mellitt, K.D. Burman, L. Wartofsky and R.S. Swerdloff (1978). Endocrinology 102: 1925-1928.

Goslings, B., H.L. Schwartz, W. Dillman, M.I. Surks and J.H. Oppenheimer (1976). Endocrinology 98: 666-675.

Goswami, A. and I.N. Rosenberg (1979). J. Biol. Chem. 254: 12326-12330.

Grant, A.M., O.M. Edwards, A.N. Howard, G.S. Challand, E.P. Wraight and I.H. Mills (1978). Clin. Endocrinol. 9: 227-231.

Green, W.L. (1976). In: "Thyroid Research". Eds. J. Robbins and L.E. Braverman, Excerpta Medica, Amsterdam, 239-243.

Green, W.L. (1978). Endocrinology 103: 826-837.

Greenbaum, A.L., K.A. Gumaa and P. McLean (1971). Arch. Biochem. Biophys. 143: 617-663.

Gross, J. and R. Pitt-Rivers (1952). Lancet I: 439-441.

Gross, J. and R. Pitt-Rivers (1953). Biochem. J. 53: 645-650.

Grussendorf, M. and M. Hüfner (1977). Clin. Chim. Acta 80: 61-66.

Hadden, D.R., T.K. Bell, D.G. McDevitt, R.G. Shanks and D.A.D. Montgomery and J.A. Weaver (1969). Acta Endocrinol. 61: 393-399.

Hagenfeldt, I., A. Melander, J. Thorell, S. Tibblin and U. Westgren (1979). Acta Chir. Scand. 145: 77-82.

Haibach, H. (1971). Endocrinology 88: 918-923.

Haigler, E.D., J.A. Pittman, Jr., J.M. Hershman and C.M. Baugh (1971). J. Clin. Endocrinol. Metab. 33: 573-581.

Harris, A.R.C., S.L. Fang, F. Azizi, L. Lipworth, A.G. Vagenakis and L.E. Braverman (1978a). Metabolism 27: 1074-1083.

Harris, A.R.C., S.L. Fang, A.G. Vagenakis and L.E. Braverman (1978b). Metabolism 27: 1680-1690.

Harris, A.R.C., S.L. Fang, J. Prosky, L.E. Braverman and A.G. Vagenakis (1978c). Endocrinology 103: 2216-2222.

Harris, A.R.C., S.L. Fang, L. Hinerfeld, L.E. Braverman and A.G. Vagenakis (1979). J. Clin. Invest. 63: 516-524.

Hartmann, K., N. Hartmann and E. Bulka (1971a). Z. Chem. 11: 344-345.

Hartmann, K., N. Hartmann and E. Bulka (1971b). Z. Chem. 11: 424-425.

Hashimoto, H. and M. Nakashima (1978). Eur. J. Pharmacol. 50: 337-347.

Hepner, G.W. and I.J. Chopra (1979). Arch. Intern. Med. 139: 1117-1120.

Hershman, J.M. (1964). J. Clin. Endocrinol. Metab. 24: 173-179.

Hershman, J.M. and L. van Middlesworth (1962). Endocrinology 71: 94-100.

Hervas, F., G. Morreale de Escobar and F. Escobar del Rey (1976). Endocrinology 98: 77-83.

Hesch, R-D., G. Brunner and H.D. Söling (1975). Clin. Chim. Acta 59: 209-213.

Hesch, R-D., B. Höffken, R. Ködding and J. Köhrle (1980). In: "Thyroid Research 1980". Eds. J.R. Stockigt and S. Nagataki. Australian Academy of Sciences and Pergamon Press, Oxford, 400-403.

Heyma, P., R.G. Larkins, J.R. Stockigt and D.G. Campbell (1978). Clin. Sci. Mol. Med. 55: 567-572.

Hillier, A.P. (1968a). J. Physiol. (London) 197: 123-134.

Hillier, A.P. (1968b). J. Physiol. (London) 197: 135-147.

Höffken, B., R. Ködding and R-D. Hesch (1977). Clin. Chim. Acta 78: 261-266.

Höffken, B., R. Ködding, J. Köhrle and R-D. Hesch (1978a). Clin. Chim. Acta 90: 45-51.

Höffken, B., R. Ködding, A. von zur Mühlen, T. Hehrmann, H. Jüppner and R-D. Hesch (1978b). Biochim. Biophys. Acta 539: 114-124.

Hoffman, W.W., D.A. Richert and W.W. Westerfeld (1966). Endocrinology 78: 1189-1197.

Hogness, J.R., T. Wong and R.H. Williams (1954). Metabolism 3: 510-517.

Hooper, M.J., J.G. Ratcliffe, W.A. Ratcliffe, J. Marshall, R.E. Young, G. Ngaei and D.H. Clark (1978). Clin. Endocrinol. 8: 267-273.

Howitt, G. and D.J. Rowlands (1966). Lancet I: 628-631.

Hsieh, A.C.L. (1962). J. Endocrinol. 25: 55-63.

Hüfner, M. and M. Grussendorf (1978). Clin. Chim. Acta 85: 243-251.

Hüfner, M. and M. Knöpfle (1976). Clin. Chim. Acta 72: 337-341.

Hüfner, M., M. Grussendorf and M. Ntokalou (1977). Clin. Chim. Acta 78: 251-259.

Inada, M., K. Kasagi, S. Kurata, K. Kazama, H. Takayama, K. Torizuka, M. Fukase and T. Soma (1975). J. Clin. Invest. 55: 1337-1348.

Ingbar, S.H. and N. Freinkel (1956). Metabolism 5: 652-666.

Ingbar, S.H. and V.A. Galton (1975). Endocrinology 96: 1525-1532.

Isaacs, J. and F. Binkley (1977a). Biochim. Biophys. Acta 497: 192-204.

Isaacs, J.T. and F. Binkley (1977b). Biochim. Biophys. Acta 498: 29-38.

Israel, Y., P.G. Walfish, H. Orrego, J. Blake and H. Kalant (1979). Gastroenterology 76: 116-122.

Jagiello, G.M. and J.M. McKenzie (1960). Endocrinology 67: 451-456.

Jennings, A.S., D.C. Ferguson and R.D. Utiger (1979). J. Clin. Invest. 64: 1614-1623.

Jirousek, L. (1968). Biochim. Biophys. Acta 170: 152-159.

Jirousek, L. and L.W. Cunningham (1968). Biochim. Biophys. Acta 170: 160-171.

Jonckheer, M.H., P. Blockx, I. Broeckaert, C. Cornette and C. Beckers (1978). Clin. Endocrinol. 9: 27-35.

Jones, S.L. and L. van Middlesworth (1960). Endocrinology 67: 855-861.

Jorgensen, E.C. (1976). Pharmacol. Ther. (B). 2: 661-682.

Kalant, H., R. Lee and E.A. Sellers (1955). Endocrinology 56: 127-134.

Kallman, B. and P. Starr (1959). Endocrinology 64: 703-706.

Kallner, G. and J.G. Ljunggren (1979). Acta Med. Scand. 206: 459-461.

Kaplan, M.M. (1979a). Endocrinology 104: 58-64.

Kaplan, M.M. (1979b). Endocrinology 105: 548-554.

Kaplan, M.M. (1980). Endocrinology 106: 567-576.

Kaplan, M.M. and R.D. Utiger (1978a). J. Clin. Invest. 61: 459-471.

Kaplan, M.M. and R.D. Utiger (1978b). Endocrinology 103: 156-161.

Kaplan, M.M., J.B. Tatro, R. Breitbart and P.R. Larsen (1979). Metabolism 28: 1139-1146.

Kassenaar, A., L.D.F. Lameyer and A. Querido (1959). Acta Endocrinol. 32: 575-578.

Kehlet, H., P.V. Klauber and J. Weeke (1979). Clin. Endocrinol. 10: 131-136.

Kempson, S., G.V. Marinetti and A. Shaw (1978). Biochim. Piophys. Acta 540: 320-329.

Klebanoff, S.J. (1967). J. Exp. Med. 126: 1063-1078.

Klebanoff, S.J. and R.A. Clark (1977). J. Lab. Clin. Med. 89: 675-686.

Klebanoff, S.J. and W.L. Green (1973). J. Clin. Invest. 52: 60-72.

Koerner, D., H.L. Schwartz, M.I. Surks, J.H. Oppenheimer and E.C. Jorgensen (1975). J. Biol. Chem. 250: 6417-6423.

Kozyreff, V., M.I. Surks and J.H. Oppenheimer (1971). Endocrinology 89: 749-755.

Krenning, E.P., R. Docter, H.F. Bernard, T.J. Visser and G. Hennemann (1978). FEBS Letters 91: 113-116.

Krenning, E.P., R. Docter, H.F. Bernard, T.J. Visser and G. Hennemann (1979). FEBS Letters 107: 227-230.

Krenning, E.P., R. Docter, H.F. Bernard, T.J. Visser and G. Hennemann (1980). Submitted.

Kuku, S.F., D.F. Child, S. Nader and T.R. Fraser (1975). Clin. Endocrinol. 4: 437-442.

Kumar, R.S., B.U. Musa, W.G. Appleton and J.T. Dowling (1968). J. Clin. Endocrinol. Metab. 28: 1335-1340.

Kumar, K.S., R. Walls and P. Hochstein (1977). Arch. Biochem. Biophys. 180: 514-521.

Kunos, G. (1977). Brit. J. Pharmacol. 59: 177-189.

Kurland, G.S., M.V. Krotkov and A. Stone Freedberg (1960). J. Clin. Endocrinol. Metab. 20: 35-46.

Lamberts, S.W.J. and T.J. Visser (1979). Endocrinology 104 suppl: 286.

Larsen, P.R. and R.D. Frumess (1977). Endocrinology 100: 980-988.

Larsen, P.R., T.E. Dick, B.P. Markovitz, M.M. Kaplan and T.G. Gard (1979). J. Clin. Invest. 64: 117-128.

Larsen, P.R., S.Z. Bavli, M. Castonguay and R. Jove (1980). J. Clin. Invest. 65: 675-681.

Larson, F.C. and E.C. Albright (1961). J. Clin. Invest. 40: 1132-1138.

Larson, F.C., K. Tomita and E.C. Albright (1955). Endocrinology 57: 338-344.

Lassiter, W.E. and J.B. Stanbury (1958). J. Clin. Endocrinol. Metab. 18: 903-906.

Latham, K.R., K.M. MacLeod, S.S. Papavasiliou, J.A. Martial, P.H. Seeburg, H.M. Goodman and J.D. Baxter (1978). In: "Receptors and Hormone Action", vol. III. Eds. L. Birnbaumer and B.W. O'Malley. Academic Press, New York, 75-100.

Laurberg, P. (1980). Acta Endocrinol. 94 suppl 236.

Laurberg, P. and J. Weeke (1978). Acta Endocrinol. 87: 88-94.

Laurberg, P. and J. Weeke (1980). Clin. Endocrinol. 12: 61-65.

Leaf, G. and A. Neuberger (1947). Biochem. J. 41: 280-287.

Leonard, J.L. and I.N. Rosenberg (1978a). Endocrinology 103: 274-280.

Leonard, J.L. and I.N. Rosenberg (1978b). Endocrinology 103: 2137-2144.

Leonard, J.L. and I.N. Rosenberg (1980). Endocrinology 106: 444-451.

Lim, V.S., V.S. Fang, A.I. Katz and S. Refetoff (1977). J. Clin. Invest. 60: 522-534.

Lindsay, R.J., K. Kelly and J.B. Hill (1979). Endocrinology 104: 1686-1697.

Lissitzky, S., M.T. Bénévent, M. Roques and J. Roche (1959). Bull. Soc. Chim. Biol. 41: 1329-1344.

Lissitzky, S., M. Roques and M.T. Bénévent (1961a). Bull. Soc. Chim. Biol. 43: 727-742.

Lissitzky, S., M.T. Bénévent and M. Roques (1961b). Bull. Soc. Chim. Biol. 43: 743-770.

Lissitzky, S., M.T. Bénévent and M. Roques (1961c). Biochim. Biophys. Acta 51: 407-410.

Ljunggren, J.G., G. Kallner and M. Tryselius (1977). Acta Med. Scand. 202: 459-462.

Ljunggren, J.G., C. Falkenberg and G. Savidge (1979). Acta Med. Scand. 205: 267-269.

Lumholtz, I.B., K. Siersbaek-Nielsen, J. Faber, C. Kirkegaard and Th. Friis (1978). J. Clin. Endocrinol. Metab. 47: 587-589.

Lumholtz, I.B., M. Busch-Sørensen, J. Faber, Th. Friis, C. Kirkegaard and K. Siersbaek-Nielsen (1979). Acta Med. Scand. 624: 31-34.

Maciel, R.M.B., I.J. Chopra, Y. Ozawa, F. Geola and D.H. Solomon (1979a). J. Clin. Endocrinol. Metab. 49: 399-405.

Maciel, R.M.B., Y. Ozawa and I.J. Chopra (1979b). Endocrinology 104: 365-371.

Maclagan, N.F. and D. Reid (1957). Ciba Fdn. Coll. Endocrinol. 10: 190-199.

Maclagan, N.F., W.E. Sprott and J.H. Wilkinson (1952). Lancet II: 915-916.

Maharajan, G., K.M. Etta, A. Singh, I.S. Ahuja and G.K. Ahuja (1978). Clin. Endocrinol. 9: 401-406.

Maloof, F., S. Smith and M. Soodak (1969). Mech. React. Sulfur Compounds 4: 61-68.

Marx, J.V., D.A. Richert and W.W. Westerfeld (1970). J. Med. Chem. 13: 1179-1181.

Marx, J.V., D.A. Richert, W.W. Westerfeld and W.R. Ruegamer (1971). Biochem. Pharmacol. 20: 3009-3020.

Mathur, H., R.P. Ekins, B.L. Brown, P.G. Malan and A.B. Kurtz (1979). Clin. Chim. Acta 91: 317-327.

Matsuda, A., Y. Wataya and D.V. Santi (1978). Biochem. Biophys. Res. Commun. 84: 654-659.

Mayrargue-Kodja, A., S. Bouchilloux and S. Lissitzky (1958). Bull. Soc. Chim. Biol. 40: 815-831.

Mazzaferi, E.L., J.C. Reynolds, R.L. Young, C.N. Thomas and A.F. Parisi (1976). Arch. Intern. Med. 136: 50-56.

McDevitt, D.G. (1976). Postgrad. Med. J. 52: 157-161.

McDevitt, D.G. and J.K. Nelson (1978). Brit. J. Clin. Pharmacol. 6: 233-237.

Meinhold, H. and P. Schürnbrand (1977). J. Clin. Chem. Clin. Biochem. 15: 419-424.

Meinhold, H. and P. Schürnbrand (1978). Clin. Endocrinol. 8: 493-497.

Meinhold, H. and T.J. Visser (1980). Clin. Chim. Acta (in press).

Melander, A. (1977). Acta Med. Scand. 201: 257-262.

Melmed, S., G. Kurtzman, A. Reed and J.M. Hershman (1979). Life Sci. 24: 1947-1952.

Merimee, T.J. and E.S. Fineberg (1976). Metabolism 25: 79-83.

Mertes, M.P., C.T-C. Chang, E. de Clercq, G-F. Huang and P.F. Torrence (1978). Biochem. Biophys. Res. Commun. 84: 1054-1059.

Miyai, K., T. Yamamoto, M. Azukizawa, K. Ishibashi and Y. Kumahara (1975). J. Clin. Endocrinol. Metab. 40: 334-338.

Moore, R., A.N. Howard, A.M. Grant and I.H. Mills (1980). Lancet I: 223-226.

Moreira-Andres, M.N., E.G. Black, D.B. Ramsden and R. Hoffenberg (1980). Clin. Endocrinol. 12: 249-255.

Morreale de Escobar, G. and F. Escobar del Rey (1962). Endocrinology 71: 906-913.

Morreale de Escobar, G., F. Escobar del Rey and P.L. Rodriquez (1962). J. Biol. Chem. 237: 2041-2043.

Morreale de Escobar, G., P.L. Rodriquez, T. Jolin and F. Escobar del Rey (1963). J. Biol. Chem. 238: 3508-3515.

Morris, D.R. and L.P. Hager (1966). J. Biol. Chem. 241: 3582-3589.

Moshang Jr., T., J.S. Parks, L. Baker, V. Vaidya and R.D. Utiger, A.M. Bongio-vanni and P.J. Snyder (1975). J. Clin. Endocrinol. Metab. 40: 470-473.

Mouriz, J., G. Morreale de Escobar and F. Escobar del Rey (1966). Endocrinology 79: 248-260.

Murchison, L.E., P.D. Bewsher, M.I. Chesters and W.R. Ferrier (1976). Br. J. Clin. Pharmacol. 3: 273-277.

Murchison, L.E., J. How and P.D. Bewsher (1979). Br. J. Clin. Pharmacol. 8: 581-587.

Naeije, R., N. Golstein, H. Clumeck, H. Meinhold, K.W. Wenzel and L. Vanhaelst (1978). Clin. Endocrinol. 8: 467-472.

Nakagawa, S. and W.R. Ruegamer (1967). Biochemistry 6: 1249-1261.

Nakano, M., T. Tsutsumi and Y. Ushijima (1971). Biochim. Biophys. Acta 252: 335-347.

Nakano, M., H. Fukuyama, K. Suwa and Y. Tsutsumi (1973). Proc. Soc. Exp. Biol. Med. 144: 164-167.

Nauman, J., A. Nauman and K. Roszkowska (1974). Mat. Med. Pol. 6: 178-182.

Nelson, J.K. and D.G. McDevitt (1975). Brit. J. Clin. Pharmacol. 2: 411-416.

Nicoloff, J.T., D.A. Fisher and M.D. Appleman Jr. (1970). J. Clin. Invest. 49: 1922-1929.

Nilsson, O.R., B.E. Karlberg, B. Kågedal, L. Tegler and S. Almqvist (1979). Acta Med. Scand. 206: 21-25.

Nomura, S., C.S. Pittman, J.B. Chambers Jr., M.W. Buck and T. Shimizu (1975). J. Clin. Invest. 56: 643-652.

Obregon, M.J., F. Roelfsema, G. Morreale de Escobar, F. Escobar del Rey and A. Querido (1979). Clin. Endocrinol. 10: 305-315.

O'Connell, M., D.C. Robbins, E.S. Horton, E.A.H. Sims and E. Danforth Jr. (1979). J. Clin. Endocrinol. Metab. 49: 242-246.

Oppenheimer, J.H. and W.H. Dillmann (1978). In: "Receptors and Hormone Action", vol. III. Eds. L. Birnbaumer and B.W. O'Malley. Academic Press, New York, 1-33.

Oppenheimer, J.H. and S.C. Werner (1966). J. Clin. Endocrinol. Metab. 26: 715-721.

Oppenheimer, J.H., H.C. Shapiro, H.L. Schwartz and M.I. Surks (1971). Endocrinology 88: 115-119.

Oppenheimer, J.H., H.L. Schwartz and M.I. Surks (1972). J. Clin. Invest. 51: 2493-2497.

Oppenheimer, J.H., H.L. Schwartz and M.I. Surks (1975). J. Clin. Endocrinol. Metab. 41: 319-324 and 1172-1173.

Orrego, H., H. Kalant, Y. Israel, J. Blake, A. Medline, J.G. Rankin, A. Armstrong and B. Kapur (1979). Gastroenterology 76: 105-115.

Otsuki, M., M. Dakoda and S. Baba (1973). J. Clin. Endocrinol. Metab. 36: 95-102.

Otten, M., G. Hennemann, R. Docter and T.J. Visser (1980). Metabolism (in press).

Pamenter, R.W. and G.A. Hedge (1980). Endocrinology 106: 162-166.

Papavasiliou, S.S., J.A. Martial, K.R. Latham and J.D. Baxter (1977). J. Clin. Invest. 60: 1230-1239.

Parker, A.J. and N. Kharasch (1959). Chem. Rev. 59: 583-628.

Pascual, A., M.J. Obregon and G. Morreale de Escobar (1979). Endocrinology 104: 1574-1579.

Pitt-Rivers, R., J.B. Stanbury and B. Rapp (1955). J. Clin. Endocrinol. Metab. 15: 616-620.

Pittman, C.S. and S.B. Barker (1959a). Endocrinology 64: 466-468.

Pittman, C.S. and S.B. Barker (1959b). Amer. J. Physiol. 197: 1271-1274.

Pittman, J.A., J.O. Tingley, J.F. Nickerson and S. Richardson Hill (1960). Metabolism 9: 293-295.

Pittman, C.S., V.H. Read, J.B. Chambers and H. Nakafuyi (1970). J. Clin. Invest. 49: 373-380.

Pittman, C.S., J.B. Chambers and V.H. Read (1971). J. Clin. Invest. 50: 1187-1196.

Pittman, C.S., A.K. Suda, J.B. Chambers Jr. and G.Y. Ray (1979a). Metabolism 28: 333-338.

Pittman, C.S., A.K. Suda, J.B. Chambers Jr., H.G. McDaniel, G.Y. Ray and B.K. Preston (1979b). J. Clin. Endocrinol. Metab. 48: 854-860.

Pittman, C.S., T. Shimuzu, A. Burger and J.B. Chambers Jr. (1980). J. Clin. Endocrinol. Metab. 50: 712-716.

Plaskett, L.G. (1961). Biochem. J. 78: 652-657.

Pogolotti Jr., A.L. and D.V. Santi (1977). In: "Bioorganic Chemistry", vol. I. Ed. E.E. van Tamelen. Academic Press, New York, 277-311.

Portnay, G.I., J.T. O'Brian, J. Bush, A.G. Vagenakis, F. Azizi, R.A. Arky, S.H. Ingbar and L.E. Braverman (1974). J. Clin. Endocrinol. Metab. 39: 191-194.

Premachandra, B.N. (1978). J. Clin. Endocrinol. Metab. 47: 746-750.

Prescott, R.W.G., P.P.B. Yeo, M.J. Watson, I.D.A. Johnston, J.G. Ratcliffe and D.C. Evered (1979). J. Clin. Pathol. 32: 321-324.

Rabinowitz, J.L. and E.S. Hercker (1971). Science 173: 1242-1243.

Ramirez, G., W. O'Neill, W. Jubiz and H.A. Bloomer (1976). Ann. Intern. Med. 84: 672-676.

Ramsden, D.B., A.M. Lawson, P.J. Raw and R. Hoffenberg (1974). Biochem. J. 143: 47-50.

Ramsden, D.B., H.P. Prince, W.A. Burr, A.R. Bradwell, E.G. Black, A.E. Evans and R. Hoffenberg (1978). Clin. Endocrinol. 8: 109-122.

Ramsden, D.B., T.J. Smith, W.A. Burr, E.G. Black, J.M. Lee and R. Hoffenberg (1979). J. Endocrinol. 82: 403-408.

Ranta, T. (1975). Endocrinology 96: 1566-1570.

Ranta, T. (1976). Acta Endocrinol. 82: 710-714.

Rastogi, G.K., R.C. Sawhney and K.K. Talwar (1976). Hormone Metab. Res. 8: 409-410.

Re, R.N., I.A. Kourides, E.C. Ridgway, B.D. Weintraub and F. Maloof (1976). J. Clin. Endocrinol. Metab. 43: 338-346.

Refetoff, S., R. Matalon and M. Bigazzi (1972). Endocrinology 91: 934-947.

Reinwein, D. and J.E. Rall (1965). J. Biol. Chem. 241: 1636-1643.

Reinwein, D., J.E. Rall and H.A. Durrer (1968). Endocrinology 83: 1023-1028.

Roche, J. and R. Michel (1954). Acta Endocrinol. 17: 385-393.

Roche, J., S. Lissitzky and R. Michel (1952). C. R. Acad. Sci. 234: 1228-1230.

Roche, J., R. Michel, W. Wolf and J. Nunez (1956a). Biochim. Biophys. Acta 19: 308-317.

Roche, J., R. Michel, P. Jouan and W. Wolf (1956b). Endocrinology 59: 425-432.

Rokos, H. and E. Scheiffele (1979). In: "The Chemistry and Biology of Pteridines." Ed. G.M. Brown, Elsevier, New York, 201-206.

Rosenberg, I.N. and A. Goswami (1979). J. Biol. Chem. 254: 12318-12325.

Rudolph, M., T. Sakurada, S.L. Fang, A.G. Vagenakis, L.E. Braverman and S.H. Ingbar (1978). J. Clin. Endocrinol. Metab. 46: 923-928.

Ruegamer, W.R., W.W. Westerfeld and D.A. Richert (1964). Endocrinology 75: 908-916.

Ruegamer, W.R., J.S. Warren, M. Barstow and W. Beck (1967). Endocrinology 81: 277-282.

Ruegamer, W.R., D.A. Richert and W.W. Westerfeld (1972). Endocrinology 91: 1468-1475.

Saberi, M., F.H. Sterling and R.D. Utiger (1975). J. Clin. Invest. 55: 218-223.

Samuels, H.H. (1978). In: "Receptors and Hormone Action", vol. III. Eds. L. Birnbaumer and B.W. O'Malley. Academic Press, New York, 35-74.

Sakurada, T., M. Rudolph, S.L.L. Fang, A.G. Vagenakis, L.E. Braverman and S.H. Ingbar (1978). J. Clin. Endocrinol. Metab. 46: 916-922.

Sander, E.G. (1978). In: "Bioorganic Chemistry", vol. II. Ed. E.E. van Tamelen. Academic Press, New York, 273-297.

Saunders, J., S.E.H. Hall and P.H. Sönsken (1978). Diabetologia 15: 29-32.

Schimmel, M. and R.D. Utiger (1977). Ann. Intern. Med. 87: 760-768.

Schussler, G.C. and J. Orlando (1978). Science 199: 686-687.

Schussler, G.C., F. Schaffner and F. Korn (1978). N. Engl. J. Med. 299: 510-515.

Schwartz, H.L., V. Kozyreff, M.I. Surks and J.H. Oppenheimer (1969). Nature 221: 1262-1263.

Schwartz, H.L., M.I. Surks and J.H. Oppenheimer (1971). J. Clin. Invest. 50: 1124-1130.

Shanks, R.G., D.C. Lowe, D.R. Hadden, D.G. McDevitt and D.A.D. Montgomery (1969). Lancet I: 993-994.

Sheahan, M.M., J.H. Wilkinson and N.F. Maclagan (1951). Biochem. J. 48: 188-192.

Siersbaek-Nielsen, K., C. Kirkegaard, P., Rogowiski, J. Faber, B. Lumholtz and Th. Friis (1978). Acta Endocrinol. 87: 80-87.

Silva, J.E. and P.R. Larsen (1977). Science 198: 617-620.

Silva, J.E. and P.R. Larsen (1978). J. Clin. Invest. 61: 1247-1259.

Silva, J.E., T.E. Dick and P.R. Larsen (1978a). Endocrinology 103: 1196-1207.

Silva, J.E., M.M. Kaplan, R.G. Cheron, T.E. Dick and P.R. Larsen (1978b). Metabolism 27: 1601-1607.

Singer, P.A. and J.T. Nicoloff (1973). J. Clin. Invest. 52: 1099-1107.

Singer, P.A., J.T. Nicoloff, R.B. Stein and J. Jaramillo (1978). J. Clin. Endocrinol. Metab. 47: 512-518.

Smallridge, R.C., L. Wartofsky, B.J. Green, F.C. Miller and K.D. Burman (1979). J. Clin. Endocrinol. Metab. 48: 32-36.

Smeulers, J., T.J. Visser, A.K.C. Burger, R. Docter and G. Hennemann (1979). Ned. T. Geneesk. 123: 12-15.

Smith, H.C., S.E. Robinson and C.J. Eastman (1980). Endocrinology 106: 1133-1136.

Smith, S.J., G. Bos, J. Gerbrandy, R. Docter, T.J. Visser and G. Hennemann (1978). Eur. J. Clin. Invest. 8: 99-102.

Snyder, S.M., R.R. Cavalieri, I.D. Goldfine, S.H. Ingbar and E.C. Jorgensen (1976). J. Biol. Chem. 251: 6489-6494.

Soboll, S., R. Scholz and H.W. Heldt (1978). Eur. J. Biochem. 87: 377-390.

Sorimachi, K. and H.J. Cahnmann (1979). Hormone Metab. Res. 11: 233-237.

Sorimachi, K. and J. Robbins (1977). J. Biol. Chem. 252: 4458-4463.

Sorimachi, K. and J. Robbins (1978a). Hormone Metab. Res. 10: 528-531.

Sorimachi, K. and J. Robbins (1978b). Biochim. Biophys. Acta 542: 515-526.

Sorimachi, K. and J. Robbins (1979a). Hormone Metab. Res. 11: 39-43.

Sorimachi, K. and J. Robbins (1979b). Biochim. Biophys. Acta 583: 443-453.

Sowers, J.R., H.E. Carlson, N. Brautbar and J.M. Hershman (1977). J. Clin. Endocrinol. Metab. 44: 237-241.

Spaulding, S.W., I.J. Chopra, R.S. Sherwin and S.S. Lyall (1976). J. Clin. Endocrinol. Metab. 42: 197-200.

Spector, D.A., P.J. Davis, J.H. Helderman, B. Bell and R.D. Utiger (1976). Ann. Intern. Med. 85: 724-730.

Sprott, W.E. and N.F. Maclagan (1955). Biochem. J. 59: 288-294.

Stanbury, J.B., M.L. Morris, H.J. Corrigan and W.E. Lassiter (1960). Endocrinology 67: 353-362.

Stasilli, N.R., R.L. Kroc and R. Edlin (1960). Endocrinology 66: 872-885.

Sterling, K.D., D. Bellabarba, E.S. Newman and M.A. Brenner (1969). J. Clin. Invest. 48: 1150-1158.

Sterling, K., M.A. Brenner and E.S. Newman (1970). Science 169: 1099-1100.

Sterling, K., M.A. Brenner and V.F. Saldanha (1973). Science 179: 1000-1001.

Stevenson, C., E. Silva and G. Pineda (1974). J. Clin. Endocrinol. Metab. 38: 390-393.

Suda, A.K., C.S. Pittman, T. Shimizu and J.B. Chambers Jr. (1978). J. Clin. Endocrinol. Metab. 47: 1311-1319.

Surks, M.I. and J.H. Oppenheimer (1971). J. Clin. Endocrinol. Metab. 33: 612-618.

Surks, M.I. and J.H. Oppenheimer (1977). J. Clin. Invest. 60: 555-562.

Surks, M.I., L. Schwartz and J.H. Oppenheimer (1969). J. Clin. Invest. 48: 2168-2175.

Surks, M.I., A.R. Schadlow, J.M. Stock and J.H. Oppenheimer (1973). J. Clin. Invest. 52: 805-811.

Suwa, K. and M. Nakano (1975). Proc. Soc. Exp. Biol. Med. 150: 401-406.

Suzuki, M., I. Ishikawa, S. Shimizu and K. Yamamoto (1961). Biochim. Biophys. Acta 51: 403-406.

Suzuki, H., N. Kadena, K. Takeuchi and S. Nakagawa (1979). Acta Endocrinol. 92: 477-488.

Takagi, A., Y. Isozaki, K. Kurata and S. Nagataki (1978). Endocrinology 103: 1434-1439.

Tamai, H., H. Suemastu, N. Kurokawa, M. Esaki, T. Ikemi, F. Matsuzuka, K. Kuma and S. Nagataki (1979). J. Clin. Endocrinol. Metab. 48: 54-58.

Tata, J.R. (1957). Proc. Soc. Exp. Biol. Med. 95: 362-364.

Tata, J.R. (1958). Biochim. Biophys. Acta 28: 95-99.

Tata, J.R. (1960). Biochem. J. 77: 214-226.

Tata, J.R., J.E. Rall and R.W. Rawson (1957). Endocrinology 60: 83-98.

Tateishi, N., T. Higashi, A. Naruse, K. Nakashima, H. Shiozaki and Y. Sakamoto (1977). J. Nutr. 107: 51-60.

Taurog, A. (1976). Endocrinology 98: 1031-1046.

Tevaarwerk, G.J.M., C.J. Hurst, P. Uksik and L. Reese (1979). Can. Med. Ass. J. 20: 1090-1093.

Tolis, G., H.G. Friesen, C.Y. Bowers and J.M. McKenzie (1974). Neuroendocrinology 15: 245-248.

Topliss, D.J., E.L. White and J.R. Stockigt (1980). J. Clin. Endocrinol. Metab. 50: 52-56.

Tsukui, T., T. Aizawa, T. Yamada and T. Kawabe (1978). Endocrinology 102: 1662-1669.

Tuomisto, J., P. Männisto, B.A. Lamberg and M. Linnoila (1976). Acta Endocrinol. 83: 522-527.

Turner, P. (1974). Drugs 7: 48-54.

Turner, P., K.L. Granville-Grossman and J.V. Smart (1965). Lancet II: 1316-1318.

Ushijima, Y., K. Suwa and M. Nakano (1973). Biochim. Biophys. Acta 320: 284-294.

Vagenakis, A.G., A. Burger, G.I. Portnay, M. Rudolph, J.T. O'Brian, F. Azizi, R.A. Arky, P. Nicod, S.H. Ingbar and L.E. Braverman (1975). J. Clin. Endocrinol. Metab. 41: 191-194.

Vagenakis, A.G., G.I. Portnay, J.T. O'Brian, M. Rudolph, R.A. Arky, S.H. Ingbar and L.E. Braverman (1977). J. Clin. Endocrinol. Metab. 45: 1305-1309.

VanArsdel, P.P. and R.H. Williams (1956). Amer. J. Physiol. 186: 440-444.

Van Hardeveld, C., M.J. Zuidwijk and A.A.H. Kassenaar (1979a). Acta Endocrinol. 91: 473-483.

Van Hardeveld, C., M.J. Zuidwijk and A.A.H. Kassenaar (1979b). Acta Endocrinol. 91: 484-492.

Van Middlesworth, L. (1974). In: "Handbook of Physiology", section 7: "Endocrinology", vol. III. "Thyroid". Eds. M.A. Greer and D.H. Solomon. American Physiological Society, Washington, 215-231.

Van Noorden, C.J.F., W.M. Wiersinga and J.L. Touber (1979). Hormone Metab. Res. 11: 366-370.

Van Pilsum, J.F., J.R. Boen and L. Bans (1973). Endocrinology 92: 135-140.

Veen, H.F. (1980). "De schildklierrest na strumectomie", thesis, Erasmus University, Rotterdam.

Verhoeven, R.P. (1978). "Hypercirculatie bij hyperthyreoidie", thesis, Erasmus University, Rotterdam.

Verhoeven, R.P., T.J. Visser, R. Docter, G. Hennemann and M.A.D.H. Schalekamp (1977). J. Clin. Endocrinol. Metab. 44: 1002-1005.

Vigneri, R., V. Pezzino, S. Filetti, S. Squatrito, A. Galbiati and P. Polosa (1975). Metabolism 24: 1209-1213.

Visser, T.J. (1978). Mol. Cell. Endocrinol. 10: 241-247.

Visser, T.J. (1979). Biochim. Biophys. Acta 569: 302-308.

Visser, T.J. (1980a). Biochim. Biophys. Acta 611: 371-378.

Visser, T.J. (1980b). Trends Biochem. Sci. 5: 222-224.

Visser, T.J. and S.W.J. Lamberts (1980). Acta Endocrinol. (in press).

Visser, T.J. and E. van Overmeeren (1979). Biochem. J. 183: 167-169.

Visser, T.J. and E. van Overmeeren (1980a). Biochim. Biophys. Acta 231: 246-252.

Visser, T.J. and E. van Overmeeren (1980b). (submitted).

Visser, T.J., I. van der Does-Tobé, R. Docter and G. Hennemann (1975a). Biochem. J. 150: 489-493.

Visser, T.J., N.L. van den Hout-Goemaat, R. Docter and G. Hennemann (1975b). Neth. J. Med. 18: 111-115.

Visser, T.J., I. van der Does-Tobé, R. Docter and G. Hennemann (1976a). In: "Thyroid Research". Eds. J. Robbins and L. Braverman. Excerpta Medica, Amsterdam, 235-238.

Visser, T.J., I. van der Does-Tobé, R. Docter and G. Hennemann (1976b). Biochem. J. 157: 479-482.

Visser, T.J., S.W.J. Lamberts, J.H.P. Wilson, R. Docter and G. Hennemann (1978a). Metabolism 27: 405-409.

Visser, T.J., L.M. Krieger-Quist, R. Docter and G. Hennemann (1978b). J. Endocrinol. 79: 357-362.

Visser, T.J., D. Fekkes, R. Docter and G. Hennemann (1978c). Biochem. J. 174: 221-229.

Visser, T.J., D. Fekkes, R. Docter and G. Hennemann (1979a). Biochem. J. 179: 489-495.

Visser, T.J., E. van Overmeeren, D. Fekkes, R. Docter and G. Hennemann (1979b). FEBS Letters 103: 314-318.

Walfish, P.G., H. Orrego, Y. Israel, J. Blake and H. Kalant (1979). Ann. Intern. Med. 91: 13-16.

Wartofsky, L. (1974). Hormone Res. 5: 112-128.

Wartofsky, L., R.C. Dimond, G.L. Noel, A.G. Frantz and J.M. Earll (1975). J. Clin. Endocrinol. Metab. 41: 485-490.

Wartofsky, L., K.D. Burman, R.C. Dimond, G.L. Noel, A.G. Frantz and J.M. Earll (1977). J. Clin. Endocrinol. Metab. 44: 85-90.

Wataya, Y. and D.V. Santi (1975). Biochem. Biophys. Res. Commun. 67: 818-823.

Westgren, U., A. Melander, S. Ingemansson, A. Burger, S. Tibblin and E. Wählin (1977a). Acta Endocrinol. 84: 281-289.

Westgren, U., A. Melander, E. Wählin and J. Lindgren (1977b). Acta Endocrinol. 85: 345-350.

Westgren, U., B. Ahrén, A. Burger, S. Ingemansson and A. Melander (1977c). Acta Med. Scand. 202: 89-92.

Westgren, U., B. Ahrén, A. Burger and A. Melander (1977d). Acta Endocrinol. 85: 526-530.

Westgren, U., A. Burger, K. Levin, A. Melander, G. Nilsson and U. Petterson (1977). Acta Med. Scand. 201: 269-272.

Wiersinga, W.M. and J.L. Touber (1977). J. Clin. Endocrinol. Metab. 45: 293-298.

Wilber, J.F. and R.D. Utiger (1969). J. Clin. Invest. 48: 2096-2103.

Wilkinson, J.H., W.F. Sprott, C.H. Bowden and N.F. Maclagan (1954). Biochem. J. 56: 215-222.

Willetts, P., D.N. Crossley, D.B. Ramsden and R. Hoffenberg (1979). J. Clin. Endocrinol. Metab. 49: 658-660.

Williams, D.E., I.J. Chopra, J. Orgiazzi and D.H. Solomon (1975). J. Clin. Endocrinol. Metab. 41: 354-361.

Williams, L.T., R.J. Lefkowitz, A.M. Watanabe, D.R. Hathaway and H.R. Besch Jr. (1977). J. Biol. Chem. 252: 2787-2789.

Williams, R.S. and R.J. Lefkowitz (1979). J. Cardiovasc. Pharmacol. 1: 181-189.

Wimpfheimer, C., E. Saville, M.J. Voirol, E. Danforth and A.G. Burger (1979). Science 205: 1272-1273.

Woeber, K.A. (1976a). Endocrinology 99: 887-890.

Woeber, K.A. (1976b). Endocrinology 98: 802-806.

Woeber, K.A. and B.A. Maddux (1978). J. Clin. Invest. 62: 577-584.

Wu, S-Y., I.J. Chopra, Y. Nakamura, D.H. Solomon and L.R. Bennett (1976). J. Clin. Endocrinol. Metab. 43: 682-685.

Wu, S-Y., I.J. Chopra, D.H. Solomon and L.R. Bennett (1978a). J. Clin. Endocrinol. Metab. 46: 691-697.

Wu, S-Y., A.H. Klein, I.J. Chopra and D.A. Fisher (1978b). Endocrinology 103: 235-239.

Wu, S-Y., I.J. Chopra, D.H. Solomon and D.E. Johnson (1978c). J. Clin. Endocrinol. Metab. 47: 1358-1362.

Wynn, J. (1968). Arch. Biochem. Biophys. 126: 880-891.

Wynn, J. and R. Gibbs (1962). J. Biol. Chem. 237: 3499-3505.

Wynn, J., R. Gibbs and B. Royster (1962). J. Biol. Chem. 237: 1892-1897.

Yamamoto, K. (1964a). Gen. Comp. Endocrinol. 4: 360-369.

Yamamoto, K. (1964b). Gen. Comp. Endocrinol. 4: 380-388.

Yamamoto, K., S. Shimizu and I. Ishikawa (1960). Jpn. J. Physiol. 10: 610-619.

Yamazaki, E. and D.W. Slingerland (1959). Endocrinology 64: 126-135.

Zimmerman, C.J., M. Izumi and P.R. Larsen (1978). Metabolism 27: 303-313.

SUMMARY

Thyroxine (T_4) is the main secretory product of the thyroid gland and though a small amount of 3,3',5-triiodothyronine (T_3) is also secreted, most of circulating T_3 is produced by 5'-deiodination of T_4 in peripheral tissues. Alternatively, 5-deiodination of T_4 yields 3,3',5'-triiodothyronine (reverse T_3, rT_3). T_3 is the only compound with significant biological activity. The amounts of T_3 and rT_3 produced from T_4 in the body may vary depending on the pathophysiological situation.

The aim of my studies described in the appendix papers was to obtain information about the characteristics and mechanism of thyroid hormone deiodination at the subcellular level. This would lead to a better understanding of the regulation of this process in the intact organism.

In chapter 1 the biosynthesis of thyroid hormone and the role of peroxidase is described. Reactions resulting in the formation of iodothyronines, i.e. iodination and coupling of tyrosine residues, follow a radical mechanism and are inhibited by derivatives of thiourea, e.g. propylthiouracil (PTU). In addition, structure-activity relationships of iodothyronines as well as pathways for the metabolism of these compounds are considered, showing that both quantitatively and qualitatively deiodination is the most important transformation of T_4.

Chapter 2 deals with the economy of thyroid hormone deiodination in physiological conditions and the role of the diet and the effects of drugs. Under normal conditions approximately 80% of T_3 and 95% of rT_3 in the human body are produced by peripheral deiodination of T_4. However, in several non-physiological situations (e.g. starvation, systemic illness and administration of drugs such as dexamethasone, propranolol, PTU and radiographic contrast agents) T_3 formation and rT_3 degradation are impaired. Supposedly, these effects are due to a diminished 5'-deiodinase activity of the tissues. The supply and utilization of glucose appears to be important in this respect.

Chapter 3 and the appendix papers concern the study of enzymatic 5- and 5'-deiodination of several iodothyronines in tissue preparations, notably liver and kidney homogenates. I studied the conversion of T_4 into T_3 and rT_3 and their further deiodination to 3,3'-diiodothyronine (3,3'-T_2) in rat liver homogenates and microsomal fractions. This included i) measurement of the metabolites with specific radioimmunoassays, ii) investigation of the number and properties of the enzymes involved with the sequential deiodination of T_4, iii) the search for possible cofactors, and iv) attempts to elucidate the mechanism of deiodination.

It is demonstrated that a single enzyme mediates the 5'-deiodination of T_4, rT_3 and $3',5'-T_2$. This same enzyme may also catalyse - albeit with low efficacy - the 5-deiodination of $3,5-T_2$. However, it seems likely that there exists a second enzyme being more specific for 5-deiodinations. Up to now efforts to separate 5- from 5'-deiodinase have been unsuccessful.

Thyroid hormone-deiodinating enzymes require mercapto compounds for activity and are strongly inhibited by derivatives of thiouracil. From the kinetics of stimulation by thiols and inhibition by thiouracil it is concluded that enzymatic deiodination follows a ping-pong mechanism. This mechanism is thought to be composed of two half-reactions. In the first, an iodinium ion is transferred from the substrate to the essential sulfhydryl group of the enzyme, yielding monodeiodinated substrate and an enzyme-sulfenyl iodide complex. This enzyme intermediate is reduced in the second half-reaction by thiol groups of the cofactor with the formation of free enzyme and iodide.

In chapter 4 an attempt has been made to relate in vivo observations of an altered thyroid hormone metabolism to the characteristic features of enzymatic deiodination. It is proposed that reduced glutathione is the endogenous cofactor and that a decrease in the glutathione sulfhydryl-disulfide ratio is responsible for the impairment of tissue 5'-deiodinase activity during fasting. This may be an indirect result of increased fatty acid oxidation in the liver cell in this situation. Additional regulatory mechanisms may involve effects on cellular uptake of substrate.

SAMENVATTING

Schildklierhormoon stimuleert het energieverbruik van de lichaamscellen (basaal metabolisme). Het door de schildklier geproduceerde hormoon bezit echter weinig biologische aktiviteit, omdat veel van de inaktieve voorloper thyroxine $(T_4)^*$ en slechts weinig 3,3',5-trijoodthyronine $(T_3)^*$ gesecerneerd wordt. T_3 is de enige vorm van schildklierhormoon met significante biologische aktiviteit. Een groot gedeelte van het circulerende T_3 ontstaat bij de dejodering van T_4 in de lever en de nieren. Bij dit proces kan ook 3,3',5'-trijoodthyronine ("reverse" T_3, rT_3)* gevormd worden, een metaboliet met verwaarloosbare hormonale aktiviteit.

Recent is aangetoond dat de hoeveelheid T_4 die in T_3 dan wel rT_3 wordt omgezet niet konstant is. Wellicht wordt dit bepaald door de behoefte van het lichaam aan meer of minder biologisch aktief schildklierhormoon.

Mijn onderzoek had tot doel de eigenschappen van de enzymen te bestuderen die betrokken zijn bij de dejodering van schildklierhormoon en het mechanisme van de dejoderingsreakties op te helderen. Deze onderzoekingen zijn in de bijgevoegde publikaties beschreven. Met dit proefschrift werd beoogd:

i) een literatuur-overzicht te geven betreffende de bestudering van de dejodering van T_4 in het intakte organisme;

ii) mijn onderzoek en dat van anderen naar de werking van bovengenoemde enzymen te bespreken;

iii) de manier waarop de dejodering van schildklierhormoon in het lichaam wordt geregeld te verklaren aan de hand van onder ii) verkregen resultaten.

Sommige geneesmiddelen (verbindingen afgeleid van thiouracil) remmen zowel de aanmaak (jodering) als de afbraak (dejodering) van schildklierhormoon. Dit zou kunnen wijzen op mogelijke overeenkomsten tussen beide processen. Vandaar dat in hoofdstuk 1 wordt beschreven hoe de joodthyronines in de schildklier worden vervaardigd.

Het schildkliereiwit thyroglobuline bevat meerdere exemplaren van het aminozuur tyrosine. Deze kunnen worden gejodeerd en vervolgens samengevoegd tot joodthyronines. Zowel de joderings- als de koppelingsreaktie worden uitgevoerd door hetzelfde enzym: het schildklier-peroxidase. Vroeger is verondersteld dat hierbij een bepaald jodium-enzym complex (zgn. sulfenyl jodide) wordt gevormd. Thiouracil zou met dit complex reageren en daarmee de vorming van het hormoon belemmeren. Dit lijkt echter niet waarschijnlijk.

---

*het cijfer duidt op het aantal jodium atomen in het molecuul (zie figuur 1.5.)

Dat dejodering van het T_4-molecuul een belangrijke reaktie is wordt nog eens onderstreept door de gegevens die verder in hoofdstuk 1 worden besproken. Het is waarschijnlijk zelfs zo dat elk effekt van T_4 toediening aan mensen maar ook aan proefdieren te verklaren is doordat uit dit T_4 in het lichaam het aktieve T_3 wordt gevormd.

De herkomst van circulerend T_3 en rT_3 is op verschillende manieren onderzocht (hoofdstuk 2). De konklusie is dat ongeveer 80% van het T_3 en zelfs 95% van het rT_3 ontstaan bij de dejodering van T_4 en, dus, dat slechts 20% van het T_3 en 5% van het rT_3 direkt door de schildklier aan de bloedbaan worden afgescheiden.

Dejodering van T_4 gebeurt niet willekeurig. In diverse omstandigheden (zoals tijdens vasten, ziekten en in gevallen van "stress") maar ook na toediening van bepaalde medicamenten (waaronder weer het thiouracil) blijkt het T_3-gehalte in het bloed verlaagd te zijn en het rT_3-gehalte verhoogd, terwijl het T_4 meestal niet veel verandert. (Langere toediening van thiouracil leidt uiteraard ook tot een verlaging van de T_4 spiegels). De lage T_3 spiegels blijken het gevolg te zijn van een verminderde omzetting van T_4 in T_3. Echter de produktie van rT_3 uit T_4 blijkt niet veranderd te zijn. Dat het rT_3-niveau in het bloed is verhoogd wordt veroorzaakt door een verminderde klaring van deze stof.

Naar aanleiding van deze bevindingen heeft men verondersteld dat het enzym dat T_4 in T_3 omzet (5'-dejodase)[*] niet gelijk is aan het enzym dat de omzetting van T_4 in rT_3 bewerkstelligt (5-dejodase)[*]. Het lijkt echter waarschijnlijk dat het 5'-dejodase ook verantwoordelijk is voor de afbraak van rT_3. De bovengenoemde veranderingen in het metabolisme van schildklierhormoon kunnen dan worden verklaard door een slechter funktioneren van dit 5'-dejodase, terwijl het 5-dejodase ongemoeid verder dejodeert.

Het blijkt dat de samenstelling van het dieet - vooral de hoeveelheid koolhydraat daarin - een belangrijke rol speelt in de regulatie van de aktiviteiten van deze dejoderende enzymen. Aangezien vooral bij lever- en nierpatienten afwijkingen optreden in de spiegels van T_3 en rT_3 in het bloed, wordt verondersteld dat de dejoderingsreakties hoofdzakelijk in deze organen plaatsvinden.

Sinds kort is het mogelijk de diverse dejoderingsreakties, die leiden tot de vorming én afbraak van T_3 en rT_3, te bestuderen in weefselpreparaten waarin de celstruktuur niet meer aanwezig is (homogenaten). Voor mijn onderzoek heb ik homogenaten van rattelever gebruikt (hoofdstuk 3 en bijgevoegde publikaties). De betrokken enzymen blijken niet vrij in de cel voor te komen, maar zijn ge-

---

[*] 5 en 5' duiden op de posities van de jodiumatomen die door het enzym worden verwijderd (zie figuur 1.5.); deze zijn equivalent aan, resp., posities 3 en 3'.

bonden aan membraanstrukturen binnen de cel. Zuivering van deze enzymen is dan ook niet eenvoudig. Wel is komen vast te staan dat inderdaad één enzym (het 5'-dejodase) verantwoordelijk is voor zowel de aanmaak van T_3 (uit T_4) als de afbraak van rT_3. Tot nu toe is het echter nog niet mogelijk geweest echt te bewijzen dat bij de produktie van rT_3 uit T_4 een ander enzym (het 5-dejodase) betrokken is, hoe aannemelijk dit ook mag zijn.

De enzymen die schildklierhormoon dejoderen werken alleen in aanwezigheid van een verbinding die SH-groepen bevat (cofactor). Dejodering wordt echter geblokkeerd door thiouracil. De manier waarop de cofactor de dejodering stimuleert en thiouracil deze remt doet vermoeden dat de dejoderingsreaktie als volgt verloopt: er wordt een jodium atoom in b.v. het T_4-molecuul vervangen door een waterstofatoom waarbij T_3 of rT_3 ontstaat. Het jodiumatoom (of liever het I^+ ion) wordt overgedragen op een SH-groep van het enzym waarbij hier wel (in tegenstelling tot de joderingsreaktie) een sulfenyl jodide (SI)-groep wordt gevormd. De SI-groep op het enzym wordt vervolgens weer omgezet in de oorspronkelijke SH-groep na reaktie met de cofactor. De remmende werking van thiouracil kan op deze manier worden verklaard door reaktie met een enzym-SI-complex.

In hoofdstuk 4 wordt getracht met summiere gegevens betreffende de werking van deze enzymen een verklaring te geven voor de beïnvloeding van de dejoderingsprocessen in het lichaam onder verschillende kondities. Vooral de tijdens vasten waargenomen veranderingen hebben veel aandacht gekregen.

Dejoderingsenzymen zijn dus afhankelijk van SH-verbindingen. Aangezien gereduceerd glutathion de in hoogste concentratie voorkomende SH-verbinding in de cel is, wordt aangenomen dat dit de fysiologische cofactor is voor deze reakties. Nu blijkt dat tijdens vasten de dejoderingsaktiviteit van de cel tegelijkertijd met de concentratie van gereduceerd glutathion afneemt. Het belang van voldoende koolhydraat in het dieet voor een optimale dejoderingsaktiviteit van de cel is al genoemd. De concentratie van gereduceerd glutathion wordt echter eerder bepaald door eiwit-inname. Het is dan ook zeer waarschijnlijk dat meerdere faktoren een rol spelen in de regulatie van de dejoderingsprocessen.

Een intrigerend fenomeen blijft het feit dat tot nu toe alleen variaties in 5'-dejodase aktiviteit zijn waargenomen in tegenstelling tot het konstant blijven van de 5-dejodase aktiviteit. De konklusie is dan ook voorlopig dat, wil men meer weten omtrent de regulering van de dejoderingsreakties in het lichaam, het noodzakelijk is een dieper inzicht te krijgen in de werking van de daarbij betrokken enzymen. Hiervoor is het nodig te beschikken over zuivere enzympreparaten.

CURRICULUM VITAE

De schrijver van dit proefschrift werd in 1949 te Rotterdam geboren.
In 1967 werd het diploma HBS-B behaald aan de christelijke HBS en MMS
Charlois te Rotterdam. In hetzelfde jaar begon hij met de scheikundestudie
aan de Technische Hogeschool te Delft, alwaar hij in 1972 het doctoraal
examen met als hoofdvak organische chemie aflegde. Sinds 1973 is hij als
wetenschappelijk medewerker verbonden aan de afdeling Inwendige Genees-
kunde III van het Academisch Ziekenhuis Dijkzigt te Rotterdam.

APPENDIX PAPERS
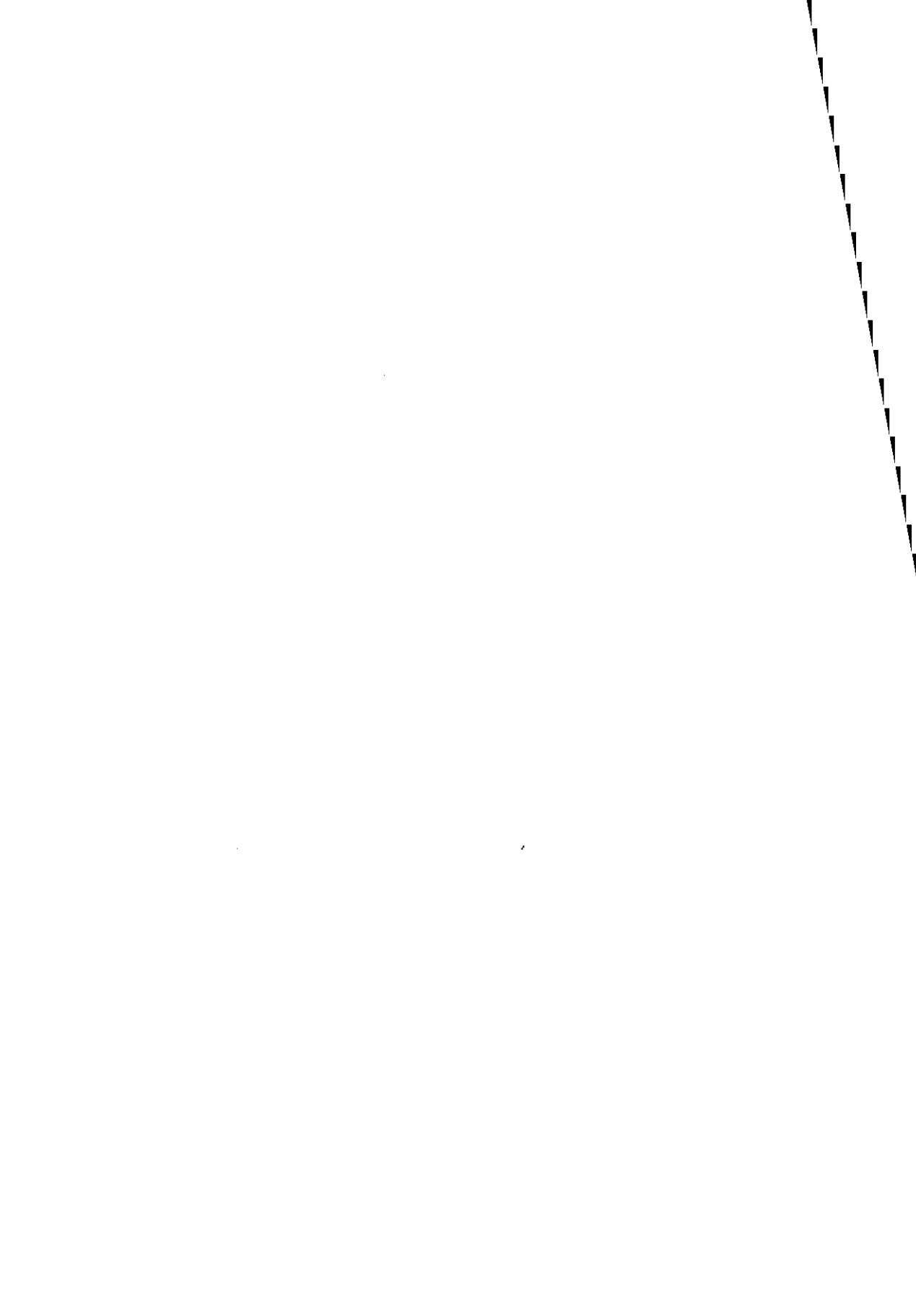
Conversion of Thyroxine into Tri-iodothyronine by Rat Liver Homogenate

By THEO J. VISSER, INEKE VAN DER DOES-TOBÉ, ROEL DOCTER
and GEORG HENNEMANN
*Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty,
Erasmus University, Rotterdam, The Netherlands*

By using a highly specific radioimmunoassay the formation of tri-iodothyronine by the deiodination of thyroxine was studied in rat liver homogenate. Several observations suggest that the reaction observed is enzymic in nature. Pre-heating the homogenate for 30 min at 56°C completely abolished conversion of thyroxine into tri-iodothyronine; the component of rat liver homogenate responsible could be saturated with substrate; iodotyrosines displayed competitive activity. Between 0° and 37°C, the tri-iodothyronine-production rate was positively correlated with incubation temperature. The addition of NAD$^+$ enhanced conversion into tri-iodothyronine, which suggests that an oxidative mechanism is involved. 5-Propyl-2-thiouracil and 6-propyl-2-thiouracil, both known to prevent deiodination *in vivo*, greatly decreased the deiodination activity of rat liver homogenate.

Since the discovery of 3,3′,5-tri-iodothyronine in human plasma (Gross & Pitt-Rivers, 1952) an abundance of evidence has been presented which clearly shows that in the circulation in humans (e.g. Pitt-Rivers *et al.*, 1955; Braverman *et al.*, 1970; Pittman *et al.*, 1971; Sterling *et al.*, 1970) and rats (e.g. Schwartz *et al.*, 1971) it is mainly derived by the peripheral deiodination of thyroxine. These observations have repeatedly been confirmed by studies *in vitro* on perfused rat heart (Rabinowitz & Hercker, 1971), cultured human liver and kidney cells (Sterling *et al.*, 1973), cultured human fibroplasts (Refetoff *et al.*, 1972) and human leucocytes (Klebanoff & Green, 1973; Woeber & Ingbar, 1973). No clear evidence that an enzymic reaction is involved in the mechanism of this conversion is as yet at hand. In rats a role for peroxidase in thyroxine deiodination has been proposed (Galton & Ingbar, 1963; Dawber *et al.*, 1971), as well as the involvement of reduced flavin nucleotide as a cofactor in the deiodination of di-iodotyrosine (Rosenberg & Ahn, 1969). These two supposed enzymic processes, however, seem to be functionally separated (Haibach, 1971). Thyroxine deiodination, in the absence of any apparent tri-iodothyronine generation, has been observed in rat skeletal-muscle homogenate stimulated by flavins (Tata, 1960), and in several other homogenized rat tissues (Galton & Ingbar, 1963). In these and several other studies only the formation of iodide and chromatographically immobile 'origin material' was observed. For instance, this was the case when deiodination was studied in chemically defined milieus, e.g. in the presence of peroxidase and H_2O_2

or H_2O_2 alone (Galton & Ingbar, 1963; Dawber *et al.*, 1971), by the combination of H_2O_2 and chelated metal ions (Reinwein *et al.*, 1968) or by illumination of a solution containing FMN (Reinwein & Rall, 1966). Almost all studies referred to have been performed with radioactive iodine-labelled thyroxine and by chromatographic analysis of the reaction products.

A different approach has been undertaken by Hesch *et al.* (1974), who applied a specific radioimmunoassay to detect tri-iodothyronine generation, when rat liver homogenate was incubated with thyroxine. We have extended these studies and the results are the subject of the present paper.

Experimental

Materials

L-Thyroxine (free acid), 3,3′,5-tri-iodo-L-thyronine, 3,5-di-iodo-L-thyronine, 3,3′,5-tri-iodothyroacetic acid, 3,3′,5-tri-iodothyropropionic acid, 3,5-dinitro-L-tyrosine, NAD$^+$, NADH, NADP$^+$ and NADPH were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., L-Tyrosine, 3-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine were from Calbiochem A.G., Lucerne, Switzerland. Tri-[^{125}I]iodo-L-thyronine (specific radioactivity approx. 500 μCi/μg) was from Abbott Laboratories, North Chicago, Ill., U.S.A.; goat anti-(rabbit γ-globulin) antiserum was from Antibodies Inc., Davis, Calif., U.S.A. 5-Propyl-2-thiouracil was synthesized by the method described by Anderson *et al.* (1945) by Dr. P. J. H. Eggels, University Hospital 'Dijkzigt', Rotterdam, The Netherlands.

Preparation of rat liver homogenate

Male Wistar rats weighing approx. 200g were used. After perfusion *in situ* through the portal vein, rat liver was minced, washed and subsequently homogenized in 3vol. of 0.25M-sucrose in 0.05M-Tris-HCl, pH7.4 (sucrose–Tris buffer) at 4°C. The homogenate was centrifuged for 5min at 2000g at 2°C. The resulting pellet was discarded.

The protein content of the homogenate was determined after solubilization in 0.1M-NaOH by using the biuret method.

Deiodination studies

Thyroxine and other substances were dissolved and diluted in sucrose–Tris buffer immediately before use and added in $10\mu l$ quantities for incubation with 1ml samples of homogenate. Control experiments were conducted in which thyroxine was incubated with sucrose–Tris buffer or with homogenate preheated for 30min at 56°C. Since these controls yielded identical results, the former was subsequently omitted. At increasing time-intervals $200\mu l$ portions of the reaction mixtures were taken and added to $400\mu l$ of ice-cold 95% (v/v) ethanol. The precipitates were spun down (1500g, 15min) and the tri-iodothyronine concentrations were measured directly in $100\mu l$ portions of the supernatants in duplicate. The recovery of added tri-[^{125}I]iodothyronine amounted to 85.8 $\pm 4.5\%$ (mean$\pm$S.D., $n = 22$).

Radioimmunoassay for tri-iodothyronine

The radioimmunoassay utilizes rabbit antiserum raised against a tri-iodothyronine–bovine serum albumin conjugate (Docter *et al.*, 1972). In Table 1 the

composition of the assay mixtures is shown. The mixtures were incubated for 16h at 4°C. In the assay $100\mu l$ of previously titred goat anti-(rabbit γ-globulin) antiserum was subsequently added to each tube and the mixtures were kept for an additional 16h at 4°C. The tubes were centrifuged and the supernatants were subsequently aspirated. The pellets were counted for radioactivity in a Packard gamma spectrometer. Non-specific binding of tri-[^{125}I]-iodothyronine to the precipitate was evaluated by preparing controls containing normal rabbit serum without antiserum. The amount of tri-[^{125}I]iodothyronine specifically bound to the antibody was expressed as a percentage of the radioactivity precipitated in the tubes without added unlabelled tri-iodothyronine, which was usually approx. 45% of the total amount of radioactivity. To calculate the tri-iodothyronine content of unknown samples the standard curve was transformed into a logit-log plot (Feldman & Rodbard, 1972).

Results and Discussion

Radioimmunoassay

Fig. 1 shows a typical radioimmunoassay standard curve. The specificity of the antiserum used was assessed by estimating the relative affinity of various thyroid-hormone analogues for the antibody (Table 2). It is concluded that the antiserum is highly specific for tri-iodothyronine, only 3,3′,5-tri-iodothyroacetic acid displaying a significant cross-reactivity. When dilutions of ethanol extracts of the reaction mixtures were assayed, the curve for antibody-bound tri-[^{125}I]iodothyronine versus log(μl of extract) (not detailed here) paralleled the standard curve. These tests establish the reliability of this assay for the

Table 1. *Composition of radioimmunoassay mixtures*

All reagents were dissolved in 0.05M-potassium phosphate buffer, pH7.4, containing 0.4% bovine serum albumin (dilution buffer). Antiserum, final dilution 1:20000 was dissolved in 2.5% normal rabbit serum in dilution buffer. Extracts of reaction mixtures were made as described in the Experimental section.

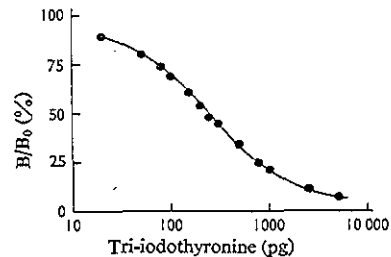|	Volume for standard curve (μl)	Volume for samples (μl)
Tri-[^{125}I]iodothyronine, 20pg	100	100
Tri-iodothyronine standard	100	
64% (v/v) Ethanol	100	
Extract of reaction mixture		100
Antiserum	100	100
Dilution buffer	600	700

Fig. 1. *Standard curve for the radioimmunoassay of tri-iodothyronine*

Conditions are detailed in the Experimental section and in Table 1. Ordinate values show the amount of tri-[^{125}I]-iodothyronine bound (B) as a percentage of radioactivity precipitated without added unlabelled tri-iodothyronine (B_0).

1975

Cross-reactivity is defined as the ratio of the concentration of tri-iodothyronine to the concentration of analogue both resulting in a 50% decrease of antibody-bound tri-[^{125}I]iodothyronine.

Compound	Cross-reactivity
3,3′,5-Tri-iodo-L-thyronine	1.0
3,3′,5,5′-Tetraiodo-L-thyronine	<0.001
3,5-Di-iodo-L-thyronine	0.003
3,3′,5-Tri-iodothyroacetic acid	0.77
3,3′,5-Tri-iodothyropropionic acid	<0.001
3-Iodo-L-tyrosine	<0.001
3,5-Di-iodo-L-tyrosine	<0.001
3,5-Dinitro-L-tyrosine	<0.001
L-Tyrosine	<0.001
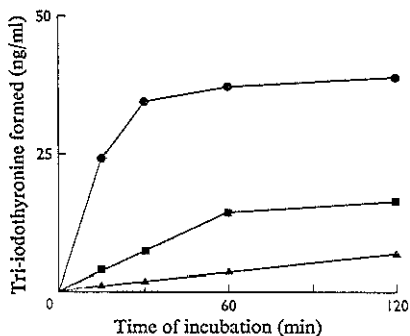
Fig. 2. *Dependence of tri-iodothyronine accumulation on incubation time and temperature*

Portions (1ml) of rat liver homogenate (43mg of protein/ml) were made to react in duplicate with 1μg of thyroxine at 0°C (▲), 22°C (■) and 37°C (●). At the times indicated 200μl samples were added to ice-cold ethanol (400μl). The resulting mixtures were processed as detailed in the Experimental section.

measurement of the conversion of thyroxine into tri-iodothyronine.

Deiodination studies

Fig. 2 shows the accumulation of tri-iodothyronine, when rat liver homogenate is incubated with 1μg of thyroxine/ml (1.3μM) at different temperatures. During the first 15min of the reaction the rate of tri-iodothyronine production was 2.5nM/min at 37°C, 0.47nM/min at 22°C and only 0.16nM/min at 0°C. No tri-iodothyronine generation was observed when the homogenate was pre-heated for 30min at 56°C, and when no thyroxine was added to the homogenate the amount of tri-iodothyronine formed was negligible.
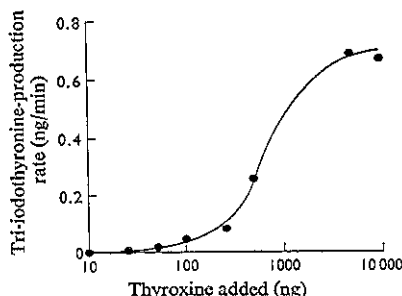

Fig. 3. *Dependence of tri-iodothyronine-production rate on thyroxine concentration*

Portions (1ml) of rat liver homogenate (51mg of protein/ml) were made to react with amounts of thyroxine as indicated in duplicate. After incubation for 15min at 37°C the reaction was stopped and the tri-iodothyronine generated was extracted by addition of ethanol as described in the Experimental section.

The relationship between the rate of tri-iodothyronine formation and the thyroxine concentration is shown in Fig. 3. Since an abundance of thyroxine- and tri-iodothyronine-binding sites exists in rat liver homogenate (e.g. Tata, 1962; Visser *et al.*, 1975), the free thyroxine concentration in the reaction system is not known and the kinetics of the reaction studied cannot be characterized simply by the standard procedure for the estimation of the Michaelis constant. At concentrations of thyroxine above 5μg/ml (6.4μM) no further increase in reaction rate is observed, however, and this suggests that either the catalysing system is saturated in these circumstances and/or the possible cofactors involved are only present in limited concentrations.

Experiments were conducted in which the effect of the addition of millimolar concentrations of nicotin-amide–adenine dinucleotides was studied (Table 3). The presence of NAD$^+$ resulted in a significant enhancement of the deiodination, whereas the addition of NADP$^+$ was without effect. An insignificant decrease in tri-iodothyronine production was observed when the incubations were performed in the presence of 1mM-NADH or 1mM-NADPH. These results could indicate that an oxidative reaction is involved.

In view of the role of flavins in deiodination reactions (Rosenberg & Ahn, 1969; Tata, 1960; Reinwein & Rall, 1966) 1mM-FAD was added to the reaction mixtures. This resulted in an approximately twofold increase in tri-iodothyronine generation. However, a considerable amount was also produced in the control tubes with pre-heated homogenate in the presence of FAD. When this experiment and the determination of

Table 3. *Effect of possible cofactors on the conversion of thyroxine into tri-iodothyronine*

Incubations were performed at 37°C as outlined in the Experimental section, by using 1 μg of thyroxine/ml. Mean values ±S.E.M. (number of experiments) are given for the amount of tri-iodothyronine formed in paired experiments conducted on separate occasions. Statistical analysis was with Student's paired t test. NS, Not significant ($P>0.05$).

Incubation conditions	Tri-iodothyronine production (ng)	
	15 min	30 min
Control	16.2±2.3 (4)	26.5±5.0 (4)
+1 mM-NAD$^+$	21.8±2.2 (4) $P<0.05$	36.7±6.2 (4) $P<0.025$
Control	18.7±0.9 (5)	29.3±1.9 (5)
+1 mM-NADH	16.5±2.4 (5) NS	26.5±3.6 (5) NS
Control	17.9±1.3 (5)	29.2±0.9 (5)
+1 mM-NADP$^+$	18.7±1.9 (5) NS	31.6±2.0 (5) NS
Control	14.7±2.3 (4)	22.0±4.0 (4)
+1 mM-NADPH	9.2±2.3 (4) NS	12.3±3.0 (4) NS

Table 4. *Effect of addition of thyroid-hormone analogues on the conversion of thyroxine into tri-iodothyronine*

For details see legend of Table 3; incubation time 15 min.

Incubation conditions	Tri-iodothyronine formed (ng)
Control	21.0±1.3 (3)
+1 μg of 3-iodotyrosine	18.9±1.3 (3) NS
Control	20.2±1.2 (4)
+10 μg of 3-iodotyrosine	9.4±2.6 (4) $P<0.05$
Control	18.3±1.5 (5)
+1 μg of 3,5-di-iodotyrosine	21.3±0.9 (5) NS
Control	18.7±2.0 (4)
+10 μg of 3,5-di-iodotyrosine	10.2±2.8 (4) $P<0.05$
Control	18.9±1.3 (4)
+1 μg of 3,5-dinitrotyrosine	18.1±1.2 (4) NS
+10 μg of 3,5-dinitrotyrosine	17.7±1.5 (4) NS
Control	19.2±1.6 (5)
+1 μg of tyrosine	19.3±0.9 (5) NS
+10 μg of tyrosine	19.3±1.0 (5) NS

Table 5. *Effect of addition of propylthiouracil on the conversion of thyroxine into tri-iodothyronine*

For details see legend to Table 3; incubation time 15 min.

Incubation conditions	Tri-iodothyronine formed (ng)
Control	16.6±2.1 (4)
+10 μM-5-Propyl-2-thiouracil	5.5±1.1 (4) $P<0.005$
+100 μM-5-Propyl-2-thiouracil	4.3±0.9 (4) $P<0.005$
Control	17.0±2.6 (4)
+10 μM-6-Propyl-2-thiouracil	5.1±2.5 (4) $P<0.005$
+100 μM-6-Propyl-2-thiouracil	3.2±1.2 (4) $p<0.001$

tri-iodothyronine by radioimmunoassay were repeated in the dark, this enhancement with FAD totally disappeared. The results of experiments without added FAD, performed in the dark, were as under normal illumination. These findings are in accordance with the work of Reinwein & Rall (1966), who detected thyroxine deiodination by illuminating a solution containing thyroxine, FMN and tracer amounts of certain cations.

The reaction was investigated in the presence of different quantities of thyroid-hormone analogues known to be unreactive in the radioimmunoassay (Table 4). Of the compounds used only 3,5-di-iodotyrosine and 3-iodotyrosine are potent inhibitors, affecting the reaction to a comparable extent. 3,5-Dinitrotyrosine was tested since it is reported to be a specific di-iodotyrosine dehalogenase inhibitor (Haibach, 1971), and this enzyme is a constituent of rat liver homogenate (Rosenberg & Ahn, 1969). The

presence of this compound, however, did not affect the reaction, from which it is concluded that the reaction proceeds via a deiodinase different from di-iodotyrosine dehalogenase. Haibach (1971) also reported on the functional separation between di-iodotyrosine deiodinase and thyroxine deiodinase in rat thyroid tissue. The addition of tyrosine to the incubation mixtures was without effect.

6-Propyl-2-thiouracil and 5-propyl-2-thiouracil, when administered to rats (Oppenheimer *et al.*, 1972; Bernal & Escobar del Rey, 1974) or humans (Hershman, 1964; Saberi *et al.*, 1975; Geffner *et al.*, 1975), prevent peripheral deiodination of thyroxine and tri-iodothyronine besides displaying goitrogenic activity. Another goitrogen, methimazole, leaves the conversion of thyroxine into tri-iodothyronine unaffected. These goitrogens exert their anti-thyroid activity mainly by inhibiting oxidative iodination of tyrosine by thyroidal peroxidase (Astwood, 1949). 5-Propyl-2-thiouracil and 6-propyl-2-thiouracil were tested for their potential inhibitory effect on the deiodination. Table 5 shows that propylthiouracil strongly impairs the conversion of thyroxine into tri-iodothyronine. Addition of methimazole in equimolar concentrations was without effect (T. J. Visser & I. van der Does-Tobé, unpublished work). From the

1975

experiments involving NAD$^+$, 5-propyl-2-thiouracil and 6-propyl-2-thiouracil we suggest that thyroxine is deiodinated by rat liver homogenate, resulting in a production of tri-iodothyronine, by an oxidative mechanism.

Preliminary experiments showed that the catalysing activity is predominantly associated with the microsomal fraction (T. J. Visser & I. van der Does-Tobé, unpublished work). Whether the deiodination occurs at random, resulting in a concomitant production also of 3,3′,5′-tri-iodothyronine, and whether 3,3′,5-tri-iodothyronine itself is also deiodinated by the same enzyme or exhibits some kind of product inhibition remains to be established.

References

Anderson, G. W., Halverstadt, I. F., Miller, W. H. & Robbin, R. O. (1945) *J. Am. Chem. Soc.* **67**, 2197

Astwood, E. B. (1949) *Ann. N.Y. Acad. Sci.* **50**, 419–443

Bernal, J. & Escobar del Rey, F. (1974) *Acta Endocrinol. (Copenhagen)* **77**, 276–281

Braverman, L. E., Ingbar, S. H. & Sterling, K. (1970) *J. Clin. Invest.* **49**, 855–864

Dawber, N. A., Galton, V. A. & Ingbar, S. H. (1971) *Endocrinology* **88**, 144–148

Docter, R., Hennemann, G. & Bernard, H. (1972) *Isr. J. Med. Sci.* **8**, Abstr. 1870

Feldman, H. & Rodbard, D. (1972) in *Principles of Competitive Protein Binding Assays* (Odell, D. W. & Daughaday, W. H., eds.), pp. 158–173, J. B. Lippincott Co., Philadelphia and Toronto

Galton, V. A. & Ingbar, S. H. (1963) *Endocrinology* **73**, 596–605

Geffner, D. L., Azukizawa, M. & Hershman, J. M. (1975) *J. Clin. Invest.* **55**, 224–229

Gross, J. & Pitt-Rivers, R. (1952) *Lancet* i, 439–441

Haibach, H. (1971) *Endocrinology* **88**, 918–923

Hershman, J. M. (1964) *J. Clin. Endocrinol. Metab.* **24**, 173–179

Hesch, R. D., Brunner, G. & Söling, D. (1974) *Endocrinol. Exp.* **8**, Abstr. 212

Klebanoff, S. J. & Green, W. L. (1973) *J. Clin. Invest.* **52**, 60–72

Oppenheimer, J. H., Schwartz, H. L. & Surks, M. I. (1972) *J. Clin. Invest.* **51**, 2493–2497

Pittman, C. S., Chambers, J. B., Jr. & Read, V. H. (1971) *J. Clin. Invest.* **50**, 1187–1196

Pitt-Rivers, R., Stanbury, J. B. & Rapp, B. (1955) *J. Clin. Endocrinol. Metab.* **15**, 616–620

Rabinowitz, J. L. & Hercker, E. S. (1971) *Science* **173**, 1242–1243

Refetoff, S., Matalon, R. & Bigazzi, M. (1972) *Endocrinology* **91**, 934–947

Reinwein, D. & Rall, J. E. (1966) *J. Biol. Chem.* **241**, 1636–1643

Reinwein, D., Rall, J. E. & Durrer, H. A. (1968) *Endocrinology* **83**, 1023–1028

Rosenberg, I. N. & Ahn, C. S. (1969) *Endocrinology* **84**, 727–737

Saberi, M., Sterling, F. H. & Utiger, R. D. (1975) *J. Clin. Invest.* **55**, 218–223

Schwartz, H. L., Surks, M. I. & Oppenheimer, J. H. (1971) *J. Clin. Invest.* **50**, 1124–1125

Sterling, K., Brenner, M. A. & Newman, E. S. (1970) *Science* **169**, 1099–1100

Sterling, K., Brenner, M. A. & Saldanha, V. F. (1973) *Science* **179**, 1000–1001

Tata, J. R. (1960) *Biochem. J.* **77**, 214–226

Tata, J. R. (1962) *Recent Prog. Horm. Res.* **18**, 221–268

Visser, T. J., Docter, R., Stinis, J. T., Bernard, B. & Hennemann, G. (1975) *Proc. Int. Conf. Thyroid Horm. Metab.* (Harland, W. A. & Orr, J. S., eds.), Academic Press, London, in the press

Woeber, K. A. & Ingbar, S. H. (1973) *J. Clin. Invest.* **52**, 1796–1803

Subcellular Localization of a Rat Liver Enzyme Converting Thyroxine into Tri-iodothyronine and Possible Involvement of Essential Thiol Groups

By THEO J. VISSER, INEKE VAN DER DOES-TOBÉ, ROEL DOCTER and
GEORGE HENNEMANN
*Department of Internal Medicine III and Clinical Endocrinology, Faculty of Medicine,
Erasmus University, Rotterdam, The Netherlands*

Experiments with rat liver homogenates showed that on subcellular fractionation the ability to catalyse the conversion of thyroxine into tri-iodothyronine was lost. The activity could in part be restored by addition of the cytosol to the microsomal fraction. Both components were found to be heat labile. The necessity of the presence of cytosol could be circumvented by incorporation of thiol-group-containing compounds in the medium. Optimal enzymic activity was observed in the presence of dithiothreitol and EDTA in medium of low osmolarity. By comparing the distribution of the converting enzyme over the subcellular fractions with a microsomal marker enzyme, glucose 6-phosphatase, it was demonstrated that the former is indeed of microsomal origin. Finally, it was shown that thiol groups play an essential role in the conversion of thyroxine into tri-iodothyronine.

Soon after the detection of 3,3',5-tri-iodothyronine in human plasma in 1952 by Gross & Pitt-Rivers, it was recognized that circulating tri-iodothyronine was in part derived from peripheral deiodination of thyroxine (Pitt-Rivers *et al.*, 1955; Larson *et al.*, 1955). It was suggested that tri-iodothyronine is the principal biologically active hormone, with thyroxine as a large precursor pool enabling the body to maintain constant effective thyroid hormone concentrations (Gross & Pitt-Rivers, 1954; Schwartz *et al.*, 1971). This pro-hormone function of thyroxine has been substantiated by the finding that its affinity for the nuclear thyroid hormone receptor is small relative to tri-iodothyronine (Oppenheimer *et al.*, 1973; Samuels & Tsai, 1973; Docter *et al.*, 1976).

The study of the conversion of thyroxine into tri-iodothyronine *in vitro* has been hampered by the occurrence of an apparently non-specific deiodination yielding reaction products other than tri-iodothyronine (Tata, 1960; Stanbury *et al.*, 1960; Wynn *et al.*, 1962; Galton & Ingbar, 1963; Nakagawa & Ruegamer, 1967; Schwartz *et al.*, 1969; Dawber *et al.*, 1971). Other investigations by Larson *et al.* (1955), Rabinowitz & Hercker (1971), Sterling *et al.* (1973), Klebanoff & Green (1973) and Woeber & Ingbar (1973) using tissue slices or isolated cells have unequivocally shown that production of tri-iodothyronine as a result of mono-deiodination of thyroxine can be observed.

These observations were extended by the application of specific radioimmunoassays for the measurement of tri-iodothyronine on incubation of tissue

homogenates with thyroxine by Hesch *et al.* (1975), Chiraseveenuprapund *et al.* (1975) and Visser *et al.* (1975). The mechanism of the specific deiodination, however, remains unclear. We report in the present paper results of investigations that demonstrate that the conversion of thyroxine into tri-iodothyronine is catalysed by an enzyme of the endoplasmic reticulum of rat liver tissue and that thiol groups are essential for this enzymic activity.

Experimental

Materials

L-Thyroxine, *p*-chloromercuriphenylsulphonic acid and dithiothreitol were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of rat liver homogenate and subcellular fractionation

The post-nuclear supernatant of rat (Wistar) liver homogenate was prepared in 25mM-Tris/HCl, pH7.4, containing 0.25M-sucrose as previously described (Visser *et al.*, 1975). This preparation was fractionated into mitochondrial, lysosomal, microsomal and cytosolic fractions by means of differential centrifugation essentially by the method of de Duve *et al.* (1955). The purity of the fractions thus obtained was evaluated by analysis of several marker enzymes (de Duve *et al.*, 1955). As a microsomal marker glucose 6-phosphatase (EC 3.1.3.9) was measured (de Duve *et al.*, 1955). Protein was measured as described by Lowry *et al.* (1951) with bovine serum albumin as standard.

Table 1. *Tri-iodothyronine production on incubation of thyroxine* (1 μM) *with subcellular fractions in 0.25M-sucrose/25mM-Tris/HCl, pH7.4, at 37°C*

The numbers in parentheses are the protein content (in mg/ml) of the various fractions.

Fraction	Tri-iodothyronine formed (pmol/ml per 30min)
Homogenate(14.8)	14.9
Mitochondrial(1.45)	0.0
Lysosomal(1.25)	0.3
Microsomal(3.5)	0.3
Cytosolic(9.7)	0.3
Mitochondrial(1.45)+cytosolic(7.2)	1.2
Lysosomal(1.25)+cytosolic(7.2)	1.4
Microsomal(3.5)+cytosolic(7.2)	6.0
Microsomal (after 30min at 56°C)(3.5)+cytosolic(7.2)	0.2
Microsomal(3.5)+cytosolic (after 30min at 56°C)(7.2)	0.0

Table 2. *Tri-iodothyronine production on incubation of microsomal fraction (5.45mg of protein/ml) with thyroxine (1 μM) in 0.25M-sucrose/25mM-Tris/HCl, pH7.4, at 37°C after a preincubation for 30min at 0°C in the presence or absence of thiol-group-containing compounds*

Addition	Tri-iodothyronine formed (pmol/ml)	
Incubation time (min)...	15	30
None	0.4	0.8
1 mM-Dithiothreitol	16.3	27.6
1 mM-2,3-Dimercaptopropanol	7.3	11.9
1 mM-β-Mercaptoethanol	6.9	13.3

Table 3. *Tri-iodothyronine production on incubation of thyroxine* (1 μM) *with microsomal preparations (3.8mg of protein/ml) in Tris buffered media (pH7.4) of different compositions at 37°C*

Dithiothreitol (mM)	EDTA (mM)	Sucrose (M)	KCl (M)	Tri-iodothyronine formed (pmol/ml per 30min)
0	0	0.25	0	0.5
0	5	0.25	0	5.1
1	0	0.25	0	30.8
1	5	0.25	0	49.2
5	0	0.25	0	33.9
5	0	0.25	0.4	26.3
5	0	0	0	30.2
5	5	0	0	61.3
5	5	0	0.4	19.9

Conversion studies

Thyroxine (1.0nmol in a final volume of 1 ml), was incubated with various subcellular fractions suspended in buffers of different composition as indicated. The reaction was stopped by the addition of 2vol. of 95% (v/v) ethanol and the tri-iodothyronine produced was measured by radioimmunoassay (Visser *et al.*, 1975). The thyroxine preparation used showed a relative displacement potency in this radioimmunoassay of less than 0.1%, which represents maximal values for contamination with tri-iodothyronine as well as for actual cross-reaction of thyroxine.

Results

Although conversion of thyroxine into tri-iodothyronine by rat liver homogenate could readily be demonstrated (Visser *et al.*, 1975) this catalytic activity was apparently lost on subsequent fractionation (Table 1). Activity could, however, in part be restored by combining the cytosol with the microsomal fraction and to a lesser degree with the other particulate preparations. The active factor present in the soluble fraction as well as that in the particles was found to be heat labile. Heating either cytosol or the particulate components for 30min at 56°C strongly decreased the converting activity displayed by the combination of the fractions concerned.

In the course of these experiments we noticed that on standing at 0°C for approx. 4h the homogenate lost over 50% of its converting activity. In addition a decrease in activity occurred when the homogenate was dialysed for a short time; 5ml of homogenate, dialysed against 2 litre of 0.25M-sucrose/25mM-Tris/HCl, pH7.4, for 30min at 0°C, possessed only 45% activity compared with non-dialysed material.

It was observed that the requirement for cytosol could be circumvented by including a thiol-group-containing compound in the reaction mixture, namely β-mercaptoethanol, 2,3-dimercaptopropanol or dithiothreitol, with the latter being far the most effective (Table 2).

Table 3 demonstrates to what extent tri-iodothyronine accumulated, when thyroxine was incu-

bated with microsomal preparations suspended in media varying in sucrose, EDTA, KCl and dithiothreitol concentrations. Obviously conditions are optimal if both EDTA and dithiothreitol are present. KCl (0.4M) inhibited the reaction.

In the presence of 1 mM-dithiothreitol and 5 mM-EDTA, treatment at 56°C for 30 min still abolished the catalytic activity of the microsomal fraction with respect to thyroxine conversion. This was not caused by accelerated oxidation of added dithiothreitol or cysteine residues in the microsomal proteins; it was rather a matter of denaturation, since subsequent addition of dithiothreitol failed to induce activity.

To investigate the possible involvement of thiol groups, tri-iodothyronine-directed deiodination of

Table 4. *Tri-iodothyronine production on incubation of thyroxine (1 μM) with microsomal fraction in 25 mM-Tris/ HCl/1 mM-dithiothreitol/5 mM-EDTA, pH7.4, at 37°C in the presence of thiol-group-blocking reagents or CN⁻*

Addition	Tri-iodothyronine formed (%)
None	100
3 mM-*p*-Chloromercuriphenyl-sulphonic acid	0.6
3 mM-Ag⁺	2.0
3 mM-Hg⁺	2.7
1 mM-KCN	97

Table 5. *Tri-iodothyronine production on incubation of thyroxine (1 μM) with microsomal fraction in 25 mM-Tris/ HCl/1 mM-dithiothreitol/5 mM-EDTA, pH7.4, at 37°C in the presence of possible cofactors*

Addition (1 mM)	Tri-iodothyronine formed (%)
None	100
NAD⁺	115
NADH	94
NADP⁺	105
NADPH	78

thyroxine was studied by incubating it with a microsomal preparation preincubated with agents known to become covalently linked to cysteine residues (Table 4). In the presence of *p*-chloromercuriphenylsulphonic acid, Hg⁺ or Ag⁺, tri-iodothyronine generation was almost completely suppressed. This decrease in activity was not due to reactions with dithiothreitol and the consequent decrease in its effective concentration, but rather to substitutions at the thiol groups of the enzyme. Experiments were performed in which the preincubation with the cysteine-directed inhibitors was followed by a second preincubation in the presence of excess of dithiothreitol and in no case was any restoration of activity observed. Table 4 also shows that addition of KCN to the reaction mixture did not affect the amount of tri-iodothyronine generated from thyroxine, rendering the involvement of a prosthetic haem group unlikely.

The monodeiodination of thyroxine by the microsomal fraction was investigated in the presence of millimolar concentrations of nicotinamide–adenine dinucleotides (Table 5). In no single instance was any enhancement of the reaction rate observed.

In Table 6 the subcellular distributions of the converting enzyme (assayed under optimal conditions with respect to EDTA and dithiothreitol concentrations) and glucose 6-phosphatase are compared. The highest tri-iodothyronine production rate is observed with the microsomal fraction.

Discussion

Our observations suggest that rat liver homogenate contains a diffusible factor, the presence of which is a prerequisite for the conversion of thyroxine into tri-iodothyronine. The loss of activity on keeping the homogenate at low temperatures could indicate that the factor(s) involved are inactivated either by proteolytic breakdown or, more likely, by oxidation due to exposure to air. The latter possibility was supported by the finding that inclusion of 1 mM-dithiothreitol in the incubation mixture enhanced the production of tri-iodothyronine from thyroxine as

Table 6. *Specific activities of glucose 6-phosphatase and of the enzyme converting thyroxine into tri-iodothyronine in subcellular fractions*

Tri-iodothyronine production was studied with thyroxine (1 μM) in 0.25 M-sucrose/25 mM-Tris/HCl/3 mM-dithiothreitol/ 3 mM-EDTA, pH7.4, at 37°C.

Fraction	Glucose 6-phosphatase (μmol of phosphate/min per mg of protein)	Thyroxine-5'-deiodinase (pmol of tri-iodothyronine/min per mg of protein)
Homogenate	0.102	2.90
Mitochondrial	0.036	1.73
Lysosomal	0.181	4.34
Microsomal	0.200	6.46
Supernatant	0.002	0.11

Q

catalysed by the homogenate. In addition, the decrease in activity on aging of the homogenate was less rapid in the presence of dithiothreitol than in its absence. Further, it induced converting activity by the microsomal fraction. The results of experiments described in the present paper are in favour of the assumption that the enzyme is highly sensitive to oxidation. Apparently a condition for the enzyme to display converting activity is that cysteine residues are present in the reduced state. The stimulation by cytosol in the experiments described in Table 1 may have been affected by endogenous glutathione although we cannot firmly exclude additional actions of the supernatant fraction.

The only comparable study on the occurrence of the conversion in the presence of rat liver microsomal fraction is that by Hesch et al. (1975). Since these investigators did not incorporate reductants such as thiols in their media, the conversion rate they reported is very low. Under comparable circumstances they observed that less than 0.1% of the thyroxine added was deiodinated to tri-iodothyronine, whereas we found approx. 6% (Table 3).

The results described here may also be compared with observations in other laboratories (e.g. Stanbury et al., 1960; Wynn et al., 1962; Nakagawa & Ruegamer, 1967) on the deiodination of thyroxine by rat liver microsomal fraction, apparently without concomitant tri-iodothyronine production. Although these studies are conflicting on several points, especially with regard to the properties of the catalysing factor involved in this reaction, they all have in common that CN^- brings about a diminution of the degradation of thyroxine. We, however, found that CN^- did not decrease the conversion (Table 4). The explanation for this phenomenon could be that rat liver microsomal fraction catalyses the deiodination of thyroxine in two different ways: one, a non-specific CN^--sensitive deiodination leading to products other than tri-iodothyronine and a second, CN^--insensitive deiodination specifically producing tri-iodothyronine. Green (1975) reached the same conclusion. Neither of these two activities is, however, identical with iodotyrosine deiodinase (Schwartz et al., 1969; Rosenberg & Ahn, 1969; Haibach, 1971; Visser et al., 1975).

Several coenzymes separately tested with the microsomal fraction did not result in an enhancement of tri-iodothyronine production (Table 5), although we observed a small, but significant, increase in converting activity by the addition of NAD^+ to the homogenate (Visser et al., 1975). The reason for this remains unclear. Assuming that, besides tri-iodothyronine, I^- is the only product, the reaction under investigation is clearly a reduction: thyroxine+2H → tri-iodothyronine+HI. In our particular situation dithiothreitol might supply the reducing equivalents.

References

Chiraseveenuprapund, P., Buergi, U., Goswami, A. & Rosenberg, I. N. (1975) (Abstr.) Program Int. Thyroid Conf. 7th, Boston, p.75, Excerpta Medica, Amsterdam

Dawber, N. A., Galton, V. A. & Ingbar, S. H. (1971) Endocrinology 88, 144–148

de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955) Biochem. J. 60, 604–716

Docter, R., Visser, T. J., Stinis, J. T., van den Hout-Goemaat, N. L. & Hennemann, G. (1976) Acta Endocrinol. (Copenhagen) 81, 82–95

Galton, V. A. & Ingbar, S. H. (1963) Endocrinology 73, 596–605

Green, W. L. (1975) (Abstr.) Program Int. Thyroid Conf. 7th Boston, p.74, Excerpta Medica, Amsterdam

Gross, J. & Pitt-Rivers, R. (1952) Lancet i, 439–441

Gross, J. & Pitt-Rivers, R. (1954) Recent Prog. Horm. Res. 10, 109–115

Haibach, H. (1971) Endocrinology 88, 918–923

Hesch, R. D., Brunner, G. & Söling, H. D. (1975) Clin. Chim. Acta 59, 209–213

Klebanoff, S. J. & Green, W. L. (1973) J. Clin. Invest. 52, 60–72

Larson, F. C., Tomita, K. & Albright, E. C. (1955) Endocrinology 57, 338–344

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. 193, 265–275

Nakagawa, S. & Ruegamer, W. R. (1967) Biochemistry 6, 1249–1261

Oppenheimer, J. H., Schwartz, H. I., Dillman, W. & Surks, M. I. (1973) Biochem. Biophys. Res. Commun. 55, 544–550

Pitt-Rivers, R., Stanbury, J. B. & Rapp, B. (1955) J. Clin. Endocrinol. Metab. 15, 616–620

Rabinowitz, J. L. & Hercker, E. S. (1971) Science 173, 1242–1243

Rosenberg, I. N. & Ahn, C. S. (1969) Endocrinology 84, 727–737

Samuels, H. H. & Tsai, J. S. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 3488–3492

Schwartz, H. L., Kozyreff, V., Surks, M. I. & Oppenheimer, J. H. (1969) Nature (London) 221, 1262–1263

Schwartz, H. L., Surks, M. I. & Oppenheimer, J. H. (1971) J. Clin. Invest. 50, 1124–1125

Stanbury, J. B., Morris, M. L., Corrigan, H. J. & Lassiter, W. E. (1960) Endocrinology 67, 353–362

Sterling, K., Brenner, M. A. & Saldanha, V. F. (1973) Science 179, 1000–1001

Tata, J. R. (1960) Biochem. J. 77, 214–226

Visser, T. J., van der Does-Tobé, I., Docter, R. & Hennemann, G. (1975) Biochem. J. 150, 489–493

Woeber, K. A. & Ingbar, S. H. (1973) J. Clin. Invest. 52, 1796–1803

Wynn, J., Gibbs, R. & Royster, B. (1962) J. Biol. Chem. 237, 1892–1897

Kinetics of Enzymic Reductive Deiodination of Iodothyronines

EFFECT OF pH

By THEO J. VISSER, DURK FEKKES, ROEL DOCTER and GEORG HENNEMANN
*Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty, Erasmus
University, Rotterdam, The Netherlands*

5'-Deiodination of thyroxine (yielding 3,3',5-tri-iodothyronine; reaction I) and of 3,3',5'-tri-iodothyronine (yielding 3,3'-di-iodothyronine; reaction II) and 5-deiodination of thyroxine (yielding 3,3',5'-tri-iodothyronine; reaction III) and of 3,3',5-tri-iodothyronine (yielding 3,3'-di-iodothyronine; reaction IV) as catalysed by rat liver microsomal fraction were studied at pH 6.5, 7.2 and 8.0. It was found that: (1) the K_m of reaction I was relatively independent of pH (approx. $3\,\mu M$), whereas V was highest at pH 6.5 (63 pmol of 3,3',5-tri-iodothyronine/min per mg of protein); (2) the K_m of reaction II was lowest at pH 6.5 (0.035 μM), but V was highest at pH 8.0 (829 pmol of 3,3'-di-iodothyronine/min per mg of protein); (3) thyroxine inhibited reaction II competitively; K_i values were identical at pH 6.5 and 8.0 ($1\,\mu M$); (4) for both reactions III and IV K_m was lowest and V was highest at pH 8.0. The results are compatible with the view that reactions I and II are mediated by a single enzyme (iodothyronine 5'-deiodinase) and that reactions III and IV are catalysed by a second enzyme (iodothyronine 5-deiodinase).

Deiodination is the main pathway by which thyroxine is metabolized *in vivo* (Cavalieri & Rapoport, 1977; Burman, 1978). Studies *in vitro* have demonstrated that reductive deiodination of iodothyronines is enzymic in nature and involves thiol compounds. Reduced glutathione is probably the endogenous cofactor (Visser, 1978). The primary products in this cascade of reactions are the metabolically active form of the hormone 3,3',5-tri-iodothyronine (tri-iodothyronine) and the inactive isomer 3,3',5'-tri-iodothyronine (reverse tri-iodothyronine).

Both have been observed *in vitro* to be further degraded to 3,3'-di-iodothyronine (di-iodothyronine; see Scheme 1). Evidence has been presented which suggests that all possible intermediates in the sequential deiodination of thyroxine are indeed generated (Rudolph *et al.*, 1978; Sakurada *et al.*, 1978).

We suggested that two enzymes, i.e. iodothyronine 5(3)- and 5'(3')-deiodinase, are responsible for the entire deiodination of thyroxine (Visser, 1978). This was mainly based on the pH-dependence of the several reactions and on clinical findings. The concept

3,3',5-tri-iodothyronine
(tri-iodothyronine)

3,3',5,5'-Tetraiodothyronine
(thyroxine)

3,3'-di-iodothyronine
(di-iodothyronine)

3,3',5'-tri-iodothyronine
(reverse tri-iodothyronine)
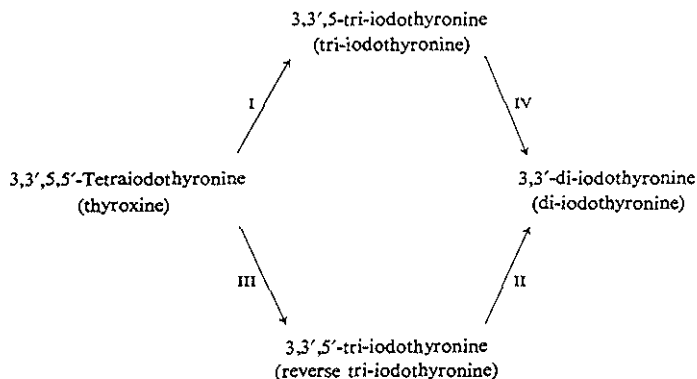
Scheme 1. *Two pathways for the sequential deiodination of thyroxine to di-iodothyronine*

is supported by the observations of competitive inhibition of reaction I by reverse tri-iodothyronine (Chopra, 1977; Hüfner *et al.*, 1977; Kaplan & Utiger, 1978) and of reaction II by thyroxine (Kaplan & Utiger, 1978). As both $k_{cat.}$ and K_m may vary with pH, one cannot adequately characterize the pH-dependence of an enzyme-catalysed reaction with measurements at a single substrate concentration. In the present study, therefore, we measured the effect of pH on V and K_m for each of the four deiodination reactions. Rat liver microsomal fraction was used as the source of the enzymes (Visser *et al.*, 1976). Evidence was obtained suggesting that reactions I and II are mediated by a single enzyme (iodothyronine 5'-deiodinase), although the interaction of reverse tri-iodothyronine with the enzyme is quite different from the reaction with thyroxine.

Experimental

Preparation of rat liver microsomal fraction in 25mM-Tris/HCl/3mM-EDTA/3mM-dithiothreitol, pH 7.4 (Visser *et al.*, 1976), and analysis of deiodination (Visser *et al.*, 1978) were done essentially as described before. Reaction mixtures consisted of 0.066M-sodium phosphate, 3mM-EDTA, 3mM-dithiothreitol, pH 6.5, 7.2 or 8.0, and substrate and microsomal fraction in the concentrations indicated. The final volume was 0.25ml. Reaction was initiated by addition of microsomal fraction and incubation was carried out at 37°C. Reaction was stopped by addition of 1ml of 1.25% of the detergent Brij 35 (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 0.06M-barbitone/0.15M-NaCl/0.1% bovine serum albumin, pH 8.6, kept at 0°C. This concentration of Brij has been shown to destroy enzymic activity completely and afforded a 100% recovery of iodothyronines. The method circumvents the use of volatile ethanol (e.g. Visser *et al.*, 1978) for the preparation of the extracts. In control experiments microsomal fraction was added after the Brij solution.

The amounts of product generated and of substrate remaining were measured in 50μl of the extracts by means of specific radioimmunoassays (Visser *et al.*, 1978). Standards were prepared in 1% Brij in 0.06M-barbitone/0.15M-NaCl/0.1% bovine serum albumin, pH 8.6.

The amount of iodothyronine measured in the control experiment was subtracted from that produced in the complete reaction mixture. Both incubations and radioimmunoassays were performed in duplicate.

The protein content of the microsomal fraction was measured after solubilization of 0.1M-NaOH, by using the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. The sources of the various materials used are given in a previous paper (Visser *et al.*, 1978). For the determination of

K_m and V values the straight lines of double-reciprocal plots were drawn by the least-squares method applied to unweighted means.

Results

Dependence of reaction rates on concentration of microsomal protein

Fig. 1 demonstrated that at pH 7.2 the production of tri-iodothyronine and di-iodothyronine from thyroxine and of di-iodothyronine from tri-iodothyronine was linear with the concentration of the microsomal fraction in the reaction mixture up to approx. 0.1 mg of protein/ml. Conversion of reverse tri-iodothyronine into di-iodothyronine reached a maximum at approx. 0.02 mg of protein/ml, owing to exhaustion of substrate under these conditions. The amount of reverse tri-iodothyronine degraded was equal to the amount of di-iodothyronine produced. Less than 10% of added thyroxine, tri-iodothyronine and di-iodothyronine (0.01 μM) was degraded by up to 0.7 mg of microsomal protein/ml. Similar findings were obtained at pH 6.5 and 8.0. In further experiments 70μg of protein/ml was used in the study of reactions I, III and IV and 3.5μg of protein/ml for reaction II.

Reaction I: 5'-deiodination of thyroxine

Double-reciprocal plots of tri-iodothyronine production rate as a function of thyroxine concentration
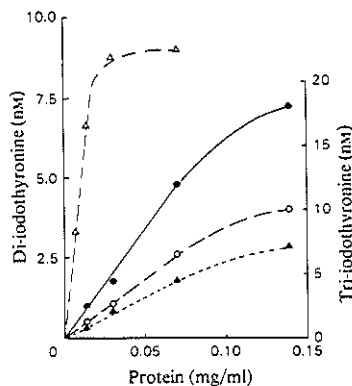

Fig. 1. *Accumulation of tri-iodothyronine* (●) *and of di-iodothyronine* (○) *from* 1 μM-*thyroxine and of di-iodothyronine from* 0.5 μM-*tri-iodothyronine* (▲) *and from* 0.01 μM-*reverse tri-iodothyronine* (∟) *as a function of microsomal protein concentration at pH 7.2*

For experimental details see the text. Incubation time was 6 min.

at three pH values are shown in Fig. 2. Apparently, the reaction obeys ordinary Michaelis–Menten kinetics with K_m values of approx. $3 \mu M$, being somewhat lower at pH 7.2 than at pH 6.5 and 8.0 (Table 1). The k_{cat} of the reaction is strongly influenced by pH, as illustrated by a 5-fold decrease in V if pH is increased from 6.5 to 8.0.

Reaction II: 5′-deiodination of reverse tri-iodothyronine

Fig. 3 shows the dependence of di-iodothyronine production on the concentration of reverse tri-iodothyronine at various pH values. Apparently, this reaction also follows Michaelis–Menten kinetics. Increasing the pH from 6.5 to 8.0 effected a 7-fold increase in K_m and a 2.5-fold increase in V (Table 1).

Reaction III: 5-deiodination of thyroxine

To obtain an accurate estimate of the rate of 5-deiodination of thyroxine the amount of reverse tri-

iodothyronine converted into di-iodothyronine has to be taken into account (see also Visser *et al.*, 1978). The implications of this procedure are illustrated in Figs. 4 and 5. At pH 6.5 no significant accumulation of reverse tri-iodothyronine was observed, whereas at pH 8.0 approximately equal amounts of reverse tri-iodothyronine and di-iodothyronine were found (Fig. 4). Production of di-iodothyronine by the pathway via tri-iodothyronine is negligible (see below and Visser *et al.*, 1978). If the sum of the concentrations of reverse tri-iodothyronine and di-iodothyronine was taken as a measure of 5-deiodination of thyroxine, this reaction was found to follow Michaelis–Menten kinetics (Fig. 5). Values for K_m were calculated to be approx. $2-3 \mu M$, being highest at pH 6.5 (Table 1). The V of this reaction is highest at pH 8.0, being about twice the value at pH 6.5 (Table 1).
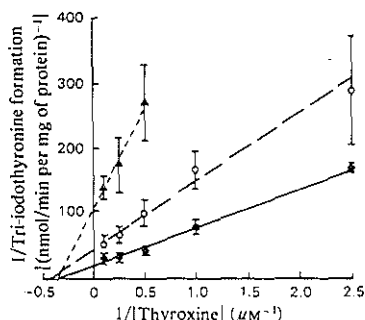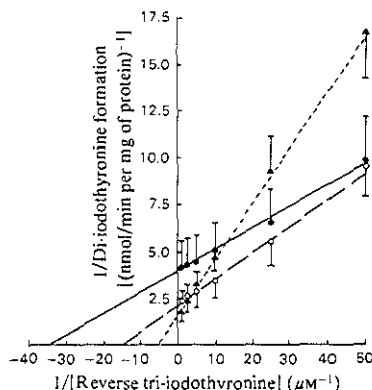

Fig. 3. *Lineweaver–Burk plot of the conversion of reverse tri-iodothyronine into di-iodothyronine at pH 6.5 (●), 7.2 (○) and 8.0 (▲) measured with a specific radioimmunoassay for the latter*
For experimental details see the text. Microsomal protein concentration was $3.5 \mu g/ml$, and incubation time 12 min. Results are means ±S.E.M. of four experiments.

Fig. 2. *Lineweaver–Burk plot of the conversion of thyroxine into tri-iodothyronine at pH 6.5 (●), 7.2 (○) and 8.0 (▲)*
For experimental details see the text. Microsomal protein concentration was 0.07 mg/ml, and incubation time 12 min. Results are means ±S.D. of five experiments.

Table 1. *Parameters of iodothyronine 5- and 5′-deiodinase-catalysed reactions in rat liver microsomal fraction*
Mean values ±S.D. are given with the numbers of experiments in parentheses.

Reaction	pH ...	K_m (μM)			V (pmol/min per mg of protein)		
		6.5	7.2	8.0	6.5	7.2	8.0
5′-Deiodination of							
thyroxine (5)		2.9 ± 0.6	2.3 ± 0.5	3.0 ± 0.6	63 ± 25	30 ± 17	13 ± 7
reverse tri-iodothyronine (4)		0.035 ± 0.010	0.064 ± 0.008	0.23 ± 0.06	342 ± 169	559 ± 230	829 ± 425
5-Deiodination of							
thyroxine (3)		3.3 ± 1.3	1.9 ± 0.4	1.8 ± 0.3	13 ± 6	18 ± 5	24 ± 2
tri-iodothyronine (5)		7.8 ± 0.9	6.2 ± 0.2	4.7 ± 1.1	21 ± 9	36 ± 7	42 ± 19

Reaction IV: 5-deiodination of tri-iodothyronine

Conversion of tri-iodothyronine into di-iodothyronine was also found to be a saturable process (Fig. 6). The K_m for this reaction was larger than for the other reactions, and was highest at pH 6.5. V was similar to that of reaction III, in both magnitude and dependence on pH.

Inhibition of 5'-deiodination of reverse tri-iodothyronine by thyroxine

The effect of thyroxine on the conversion of reverse tri-iodothyronine into di-iodothyronine was studied at pH 6.5 and 8.0. The results demonstrate that at both pH values thyroxine inhibits this reaction competitively (Fig. 7). From the change in apparent
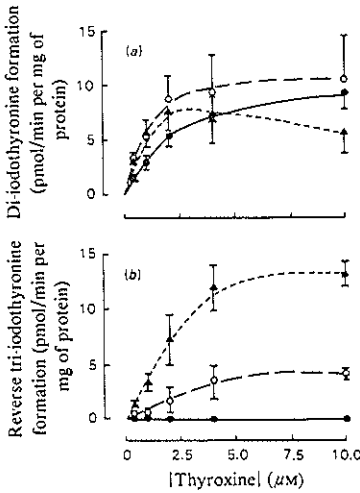
Fig. 4. *Di-iodothyronine (a) and reverse tri-iodothyronine (b) production rates as a function of thyroxine concentration at pH 6.5 (●), 7.2 (○) and 8.0 (▲)*
For experimental details see the text. Microsomal protein concentration was 0.07 mg/ml, and incubation time 12 min. Results are means ±s.d. of three experiments.
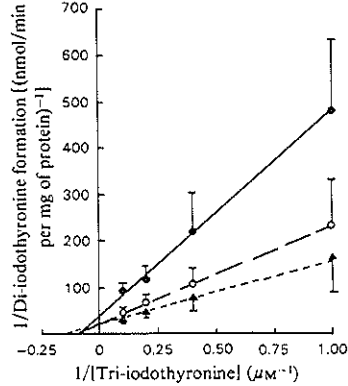
Fig. 6. *Lineweaver–Burk plot of the conversion of tri-iodothyronine into di-iodothyronine at pH 6.5 (●), 7.2 (○) and 8.0 (▲)*
For experimental details see the text. Microsomal protein concentration was 0.07 mg/ml, and incubation time 12 min. Results are means ±s.d. of five experiments.
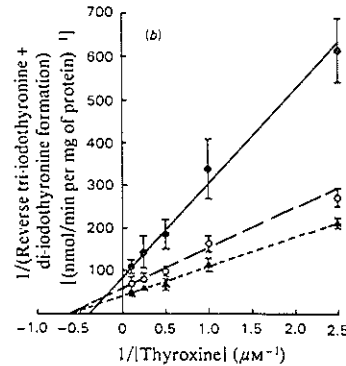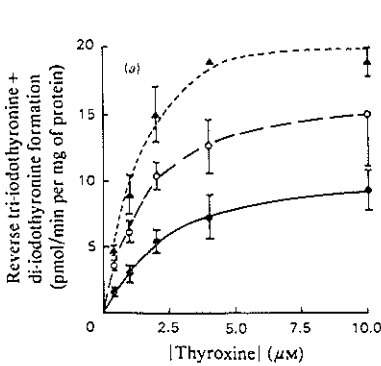
Fig. 5. *Direct (a) and double-reciprocal (b) plot of the sum of di-iodothyronine and reverse tri-iodothyronine production rates as a function of thyroxine concentration at pH 6.5 (●), 7.2 (○) and 8.0 (▲)*
For experimental details see the text and the legend to Fig. 4. Results are means ±s.d. of three experiments.

K_m for reverse tri-iodothyronine the K_i value for thyroxine was calculated to be approx. $1 \mu M$ at both pH 6.5 and 8.0 (Table 2).

Discussion

Studies *in vitro* of the enzymic reductive deiodination of iodothyronines have yielded apparently
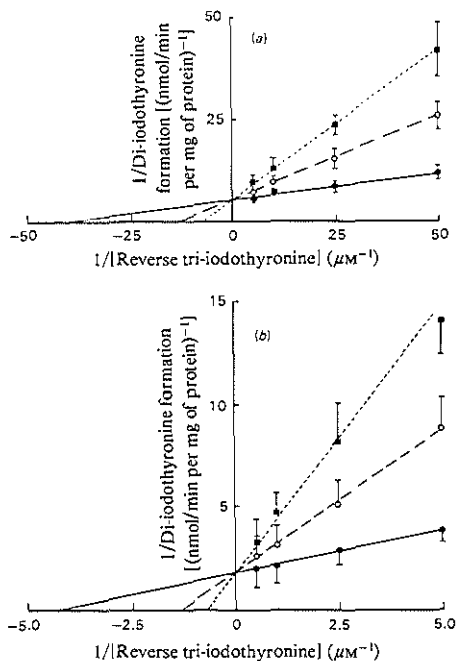

Fig. 7. *Lineweaver–Burk plot of the conversion of reverse tri-iodothyronine into di-iodothyronine at (a) pH 6.5 and (b) at pH 8.0 in the absence (●) and presence of (○) 2.5 μM- or (■) 5 μM-thyroxine*
For experimental details see the text. Microsomal protein concentration was $3.5 \mu g/ml$, and incubation time 15 min. Results are means ±s.d. of three experiments.

conflicting results for the optimum pH of these reactions. Several possibilities may explain these discrepancies. In rat liver homogenate or microsomal fraction, pH may affect the rate of deiodination indirectly by changing the availability of substrate, owing to alterations in binding to non-enzyme constituents of these preparations (Höffken *et al.*, 1977). To obtain a true evaluation of the effect of pH on reaction rates, all added substrate must be free to interact with the enzyme concerned. It is conceivable that the dependence of deiodinase activities on pH may also be influenced by the nature of the cofactor used, e.g. the pK of its thiol group. Enzymic deiodination of iodothyronines as a function of pH may further depend on the concentration of substrate tested, since K_m and $k_{cat.}$ may be affected independently. We therefore decided to study the variation in these parameters in a narrow pH-range comprising most reported optimum values. Under the conditions used in the present study, reaction rates were proportional to the microsomal protein concentration in the presence of dithiothreitol. Gross non-specific binding of substrate to the membranes is therefore excluded. If such binding did occur, then an increase in microsomal protein concentration would result not only in increased enzyme concentration but also in decreased substrate availability (Visser *et al.*, 1978). However, deviation from linearity was observed only above 0.1 mg of protein/ml.

Reaction I (5'-deiodination of thyroxine) is reported to be optimal at pH 6.0 (Höffken *et al.*, 1977) or pH 7.0 (Chopra, 1977). The K_m for this reaction is variously reported as $1.6 \mu M$ (Hüfner *et al.*, 1977), $2.5 \mu M$ (Chopra, 1977) and $7.7 \mu M$ (Kaplan & Utiger, 1978). It is demonstrated (Table 1) that reaction velocity as a function of pH at sub-saturating concentrations of substrate is determined by $k_{cat.}$.

For reaction II (conversion of reverse tri-iodothyronine into di-iodothyronine), reported values of K_m and optimum pH vary considerably. The data presented in Table 1 may at least in part explain these apparent discrepancies. It was found that both K_m and V were highly dependent on pH; K_m was lowest at pH 6.5 and V was highest at pH 8.0. Since the effect of pH on K_m is predominant, the rate of di-iodothyronine formation at low substrate concen-

Table 2. *Estimation of the K_i of the inhibition by thyroxine of the conversion of reverse tri-iodothyronine into di-iodothyronine*
K_i values were estimated by using the equation: apparent $K_m = K_m (1 + [I]/K_i)$, where the apparent K_m is $-1/$intercept on the abscissa in the Lineweaver–Burk plot (see Fig. 6) and [I] the concentration of the inhibitor.

		Apparent K_m of reverse tri-iodothyronine (μM)		K_i of thyroxine (μM)	
Concn. of thyroxine (μM)	pH ...	6.5	8.0	6.5	8.0
—		0.025	0.23	—	—
2.5		0.080	0.77	1.1	1.1
5.0		0.13	1.4	1.1	1.0

trations as used previously (Visser et al., 1978) should be highest in a slightly acidic medium, as indeed we have found. At relatively high concentrations of reverse tri-iodothyronine as used by Hüfner & Grussendorf (1978) and Chopra et al. (1978), reaction velocities would be expected to follow $k_{cat.}$. Both groups of investigators reported, in accordance with this conclusion, that conditions were optimal at approx. pH 8.0. The magnitude of the K_m at pH 7.2, 0.064 μM (Table 1), is in excellent agreement with the value published by Chopra et al. (1978), i.e. 0.065 μM. The value of this parameter at pH 7.5 as reported by Kaplan & Utiger (1978) is substantially lower (7.5 nM). This may be due to the differences in techniques, as these authors studied the disappearance of substrate on reaction of reverse tri-iodothyronine with whole rat liver homogenate. It should be emphasized, since the reaction follows a Ping Pong mechanism, that not only V but also K_m is a function of cofactor concentration (see below). This may also apply to the other reactions.

Little is known about the conversion of thyroxine into reverse tri-iodothyronine (reaction III). Hüfner et al. (1977) and Höffken et al. (1977) showed that accumulation of reverse tri-iodothyronine on incubation of thyroxine with rat liver homogenate or microsomal fraction was highest at pH 9–9.5. It has been demonstrated that this is mainly due to the fact that the high rate of reverse tri-iodothyronine removal (reaction II) occurs at a lower pH (Hüfner et al., 1977; Visser et al., 1978). If this reaction was taken into account, 5-deiodination of thyroxine was found to be optimal at pH 8.0 (Visser et al., 1978), which has also been observed by Cavalieri et al. (1977). The latter investigators studied reaction III in rat liver cytosolic fraction. This preparation contains little enzyme activity, but studies involving its use are apparently not hampered by reverse tri-iodothyronine-degrading activity (Cavalieri et al., 1977). The results in Table 1 are in agreement with the data of Cavalieri et al. (1977) and Visser et al. (1978), since K_m is lowest and V is highest at pH 8.0.

Formation of di-iodothyronine from tri-iodothyronine (reaction IV) has been shown to proceed maximally at pH 8.0 (Chopra et al., 1978; Visser et al., 1978). This appears to be due to the low K_m and high V at this pH (Table 1). The magnitude of the K_m at pH 7.2 as found in the present study, 6.2 μM, is equal to the value published by Chopra et al. (1978), i.e. 6 μM.

It has been suggested that the sequential deiodination of thyroxine is mediated by two enzymes, i.e. iodothyronine 5- and 5'-deiodinase (Visser, 1978). This hypothesis was based mainly on the finding that the reactions involving 5'-deiodination of thyroxine or reverse tri-iodothyronine showed a similar pH-dependence (Visser et al., 1978) and on the competitive inhibition by reverse tri-iodothyronine of

reaction I (Chopra, 1977; Hüfner et al., 1977; see also Kaplan & Utiger, 1978). More recent observations support this concept in that it has been shown that reaction II is inhibited competitively by thyroxine (Kaplan & Utiger, 1978). Clinical observations are in line with this hypothesis, since in several situations production of tri-iodothyronine and degradation of reverse tri-iodothyronine in peripheral tissues are simultaneously diminished (Schimmel & Utiger, 1977). We also showed that reactions involving 5-deiodination of thyroxine or tri-iodothyronine exhibit similar pH profiles (Visser et al., 1978). This is confirmed in the present paper by the demonstration of a similar dependence on pH of both K_m and V of these reactions (Table 1).

The present study, however, reveals that the influence of pH on the kinetic parameters of the 5'-deiodination reaction is dependent on the substrate used. We therefore thought it of importance to study the mutual inhibition by the substrates of reactions I and II at pH 6.5 and at pH 8.0. Since reverse tri-iodothyronine is rapidly degraded under conditions where the deiodination of thyroxine is investigated, these studies were limited to the effect of thyroxine on the 5'-deiodination of reverse tri-iodothyronine. Despite the large variation in K_m for the latter, the K_i for thyroxine was identical at pH 6.5 and 8.0 (Table 2). In concordance with Kaplan & Utiger (1978), the value of the K_i for thyroxine was close to the K_m for this compound in reaction I. It is concluded, therefore, that 5'-deiodination of thyroxine as well as of reverse tri-iodothyronine is mediated by a single enzyme.

These results strongly suggest that the presence of an iodine substituent at C-5 interferes with the interaction of the substrate with the 5'-deiodinase. Not only is the magnitude of K_m greatly increased and that of V diminished, but the dependence on pH of these parameters is changed considerably.

Recent investigations point to a Ping Pong mechanism (Laidler & Bunting, 1974) for reaction II and possibly for the deiodination of iodothyronines in general. Such a mechanism is supported by the following observations. (1) A decrease in the concentration of cofactor results in a proportional decrease in K_m and V of reaction II (T. J. Visser, unpublished work). (2) Inhibition by thiouracil is uncompetitive with respect to substrate (Chopra et al., 1978). (3) Inhibition by thiouracil is competitive with respect to cofactor (T. J. Visser, unpublished work). Thiouracil has been shown to react specifically with sulphenyl iodides, yielding mixed disulphides (Cunningham, 1964), and iodothyronine deiodinases contain essential thiol groups (Visser, 1978). Thus the reaction pathway may be regarded as a trans-iodination and the subsequent reduction of an iodo-enzyme complex (sulphenyl iodide) by cofactor. The magnitude of both V and K_m would be dependent on

which step in this pathway is rate-limiting (Laidler & Bunting, 1974). It is conceivable that in reaction I the rate-limiting step is different from that in reaction II. This may explain the difference in behaviour of both V and K_m of these reactions with respect to variations in pH.

References

Burman, K. D. (1978) *Metab. Clin. Exp.* **27**, 615–630

Cavalieri, R. R. & Rapoport, B. (1977) *Annu. Rev. Med.* **28**, 57–65

Cavalieri, R. R., Gavin, L. A., Bui, F., McMahon, F. & Hammond, M. (1977) *Biochem. Biophys. Res. Commun.* **79**, 897–902

Chopra, I. J. (1977) *Endocrinology* **101**, 453–463

Chopra, I. J., Wu, S. Y., Nakamura, Y. & Solomon, D. H. (1978) *Endocrinology* **102**, 1099–1106

Cunningham, L. W. (1964) *Biochemistry* **13**, 1629–1634

Höffken, B., Ködding, R. & Hesch, R. D. (1977) *Clin. Chim. Acta* **78**, 261–266

Hüfner, M. & Grussendorf, M. (1978) *Clin. Chim. Acta* **85**, 243–251

Hüfner, M., Grussendorf, M. & Ntokalou, M. (1977) *Clin. Chim. Acta* **78**, 251–259

Kaplan, M. M. & Utiger, R. D. (1978) *J. Clin. Invest.* **61**, 459–471

Laidler, K. J. & Bunting, P. S. (1974) *The Chemical Kinetics of Enzyme Action*, 2nd edn., Clarendon Press, Oxford

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Rudolph, M., Sakurada, T., Fang, S. L., Vagenakis, A. G., Braverman, L. E. & Ingbar, S. H. (1978) *J. Clin. Endocrinol. Metab.* **46**, 923–928

Sakurada, T., Rudolph, M., Fang, S. L., Vagenakis, A. G., Braverman, L. E. & Ingbar, S. H. (1978) *J. Clin. Endocrinol. Metab.* **46**, 916–922

Schimmel, M. & Utiger, R. D. (1977) *Ann. Intern. Med.* **87**, 760–768

Visser, T. J. (1978) *Mol. Cell. Endocrinol.* **10**, 241–247

Visser, T. J., van der Does-Tobé, I., Docter, R. & Hennemann, G. (1976) *Biochem. J.* **157**, 479–482

Visser, T. J., Fekkes, D., Docter, R. & Hennemann, G. (1978) *Biochem. J.* **174**, 221–229

BBA 68792

MECHANISM OF ACTION OF IODOTHYRONINE-5'-DEIODINASE

THEO J. VISSER

Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty, Erasmus University, Rotterdam (The Netherlands)

Summary

Production of 3,3'-di-iodothyronine (3,3'-T_2) from 3,3',5'-tri-iodothyronine (reverse T_3, rT_3) as catalysed by rat liver microsomal fraction was measured with a specific radioimmunoassay. The effect of the addition of 2-thiouracil and of varying concentrations of cofactor (dithiothreitol) on the kinetic parameters of this reaction were studied. It was found that thiouracil is an uncompetitive inhibitor with respect to substrate and a competitive inhibitor with respect to cofactor. The effect of a decrease in the concentration of cofactor was similar to the effect of addition of thiouracil, i.e. a proportional decrease in K_m and V. The results strongly suggest that enzymatic 5'-deiodination of iodothyronines follows a ping-pong mechanism, which may be envisaged as a transiodination and the subsequent reduction of the iodo-enzyme complex by cofactor. The intermediate is probably a sulfenyl iodide form of the enzyme, which reacts with thiouracil to yield a mixed disulfide.

Introduction

The observation that 2-thiouracil derivatives inhibit both enzymatic oxidative iodination of thyroglobulin in the thyroid [1] and enzymatic reductive deiodination of thyroid hormones in peripheral tissues [2] is intriguing. It suggests that thiouracil reacts with a similar intermediate in both processes. It has been proposed that in thyroid peroxidase catalysed iodinations the formation of an enzyme-sulfenyl iodide is involved [3—7]. Thioureylenes react selectively with -SI [4,6,7] groups yielding mixed disulfides. It has been shown that thyroid hormone-deiodinating enzymes (iodothyronine-5- and -5'-deiodinase) contain essential sulfhydryl groups [8—10]. Mercapto compounds such as

dithiothreitol, 2-mercaptoethanol and reduced glutathione are cofactors in this deiodination [8] (Eqn. 1, where TI_n and TI_{n-1} are substrate and mono-deiodinated iodothyronine, respectively).

$$TI_n + 2 \text{ R-SH} \rightarrow TI_{n-1} + \text{R-S-S-R} + HI \qquad (1)$$

These findings suggest that a sulfenyl iodide intermediate is involved in both iodination and deiodination of thyroid hormones. To test this hypothesis the kinetics of the conversion of 3,3′,5′-tri-iodothyronine (reverse T_3, rT_3) into 3,3′-di-iodothyronine (3,3′-T_2) (as a model for 5′-deiodinase catalysed deiodinations) were analysed. The results strongly suggest that this reaction follows a ping-pong mechanism. It is shown that thiouracil inhibits the 5′-deiodination of rT_3 uncompetitively with respect to substrate and competitively with respect to cofactor (dithiothreitol). These findings are consistent with the formation of a -SI intermediate.

Materials and Methods

Conversion of rT_3 into 3,3′-T_2 was studied as previously described [11,12]. In short, 0.01—1 μM rT_3 was reacted with rat liver microsomal fraction (3.5—7 μg of protein) in 0.25 ml 0.066 M sodium phosphate, containing 3 mM EDTA and 0.2—10 mM dithiothreitol (pH 6.5 or pH 8.0). In experiments dealing with the effect of thiouracil, this compound was added at a final concentration of 0.5 or 1 μM. After incubation for 15 min at 37°C, the reaction was halted by the addition of 1 ml 1.25% of the detergent Brij-35 in 0.06 M barbital, 0.15 M NaCl, 0.1% bovine serum albumin (pH 8.6) at 0°C. The amount of 3,3′-T_2 produced was determined radioimmunologically in 50 μl of the extract [13]. The sensitivity of this assay is approx. 1 pg (2 fmol) 3,3′-T_2/tube. Cross-reactivities by 3,3′,5-tri-iodothyronine, rT_3, 3,5- and 3′,5′-di-iodothyronine are all less than 0.05%, and by 3- and 3′-iodothyronine approx. 1%. Both incubation and radio-immunoassay were performed in duplicate. The data shown are taken from representative experiments, which were repeated at least once with similar results.

Dithiothreitol, Brij-35 and thiouracil were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; rT_3 and 3,3′-T_2 were purchased from Henning GmbH, Berlin, F.R.G.

Results

Both pH 6.0—6.5 [11] and pH 8.0 [14,15] have been reported to be optimal for the conversion of rT_3 into 3,3′-T_2. These apparently conflicting results are explained by the finding that, in the range 6.5—8.0, K_m as well as V increase with pH; from 0.035 to 0.23 μM and from 0.34 to 0.83 nmol 3,3′-T_2/min/mg protein (37°C), respectively [12]. The present investigations were, therefore, carried out both at pH 6.5 and 8.0. It has been assessed that all substrate added is free to interact with the enzyme by showing a linear relation between conversion rate and microsomal protein concentration [12].

It was found that both at pH 6.5 and 8.0 the degree of inhibition by thiouracil of the conversion of rT_3 into 3,3′-T_2 increased with substrate concen-
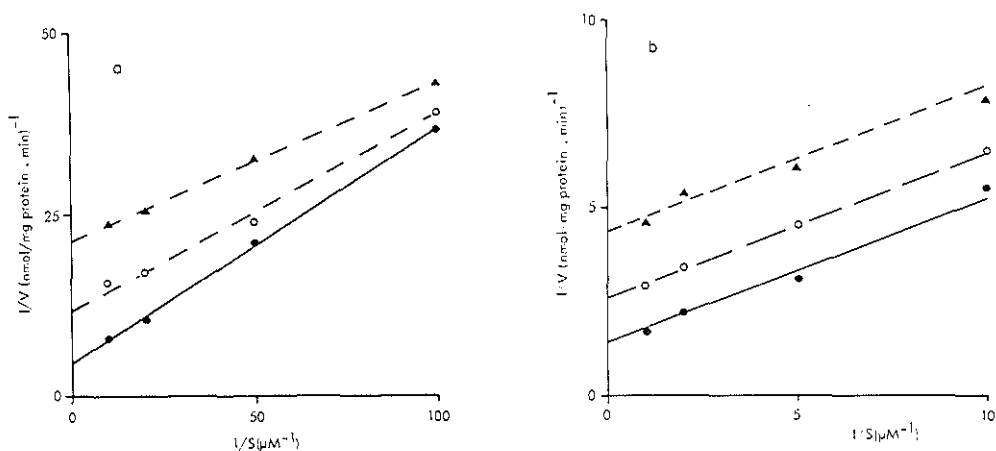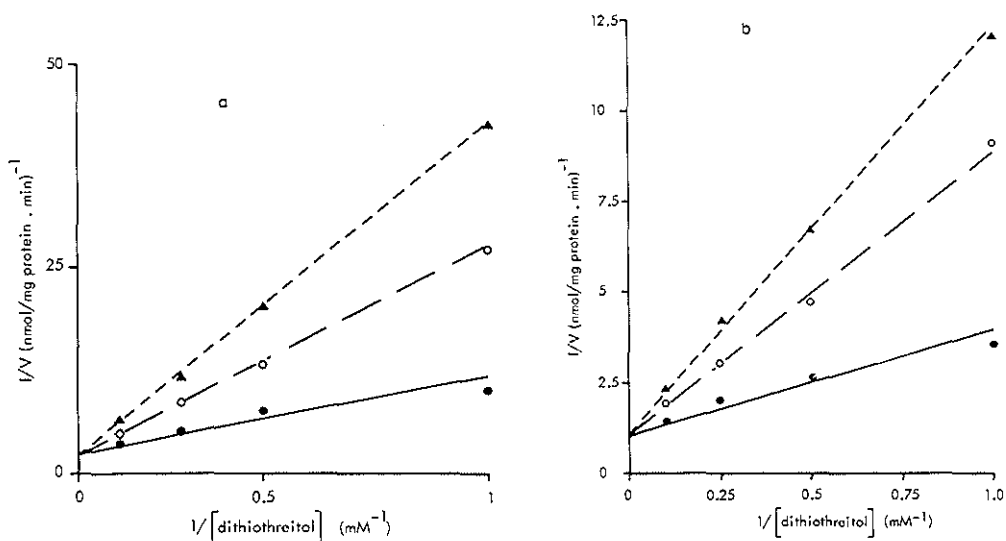
Fig. 1. Lineweaver-Burk plot of the amount of 3,3'-T$_2$ produced versus initial rT$_3$ concentration in the absence (●) and the presence of 0.5 (○) and 1 (▲) μM thiouracil at (a) pH 6.5 or (b) pH 8.0. The concentration of dithiothreitol was (a) 1 or (b) 2 mM.

tration. This is indicated by virtually parallel lines in the Lineweaver-Burk plot of the amount of 3,3'-T$_2$ produced versus initial rT$_3$ concentratin at different concentrations of thiouracil (Fig. 1), which is characteristic for uncompetitive inhibition [16]. Inhibition by thiouracil was obviated by increasing concentrations of dithiothreitol. Double-reciprocal plots of the amount of 3,3'-T$_2$ produced versus dithiothreitol concentration at different concentrations of thiouracil demonstrate (Fig. 2) that inhibition by thiouracil is competitive with respect to cofactor both at pH 6.5 and 8.0. Addition of up to 1 mM uracil did

Fig. 2. Lineweaver-Burk plot of the amount of 3,3'-T$_2$ produced versus dithiothreitol concentration in the absence (●) and the presence of 0.5 (○) and 1 (▲) μM thiouracil at (a) pH 6.5 and (b) pH 8.0. The concentration of rT$_3$ was (a) 0.1 or (b) 1 μM.
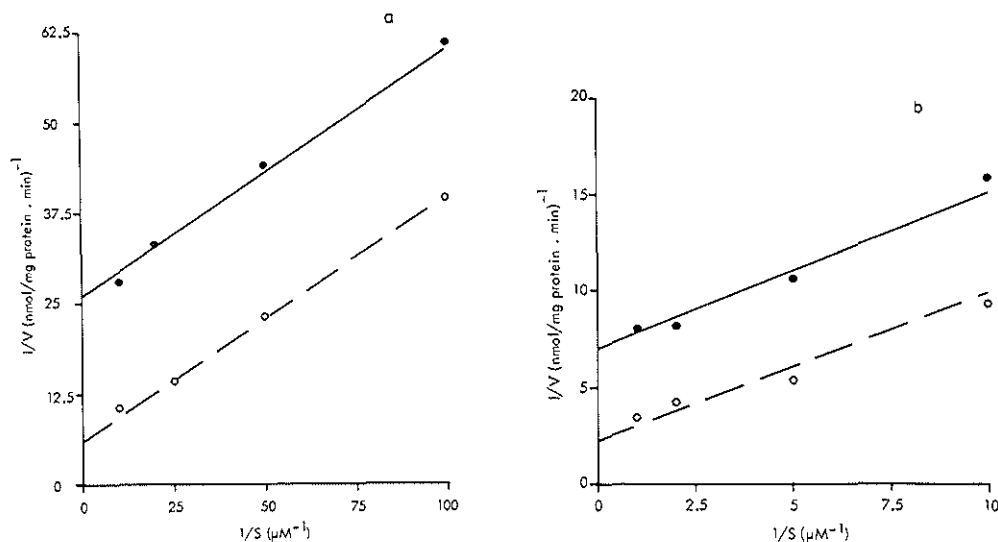
Fig. 3. Lineweaver-Burk plot of the amount of 3,3'-T$_2$ produced versus initial rT$_3$ concentration at (a) pH 6.5 or (b) pH 8.0. The concentration of dithiothreitol was 0.2 (●) or 1 (○) mM.

not affect 3,3'-T$_2$ production rate (Visser, T.J., unpublished observations). It was observed that decreasing the concentration of dithiothreitol resulted not only in decreased V values but also was there a proportional decrease in K_m. This is shown by the parallel lines in the Lineweaver-Burk plot of the amount of 3,3'-T$_2$ produced versus initial rT$_3$ concentration at different concentrations of dithiothreitol both at pH 6.5 and 8.0 (Fig. 3), which strongly suggests a ping-pong mechanism [16] for this reaction.

Discussion

The finding that inhibition by thiouracil of the 5'-deiodination of rT$_3$ is uncompetitive with respect to substrate (Fig. 1) is in accordance with data published by Chopra et al. [14] on the effect of 6-n-propyl-2-thiouracil. Chopra also found that conversion of thyroxine (T$_4$) into 3,3'-5-tri-iodo-thyronine (T$_3$) by rat liver homogenate is inhibited uncompetitively by propyl-thiouracil [9]. These observations indicate that thiouracil derivatives react with an intermediate in the enzymatic deiodination of iodothyronines [16]. The finding that the inhibition by thiouracil is competitive with respect to cofactor (Fig. 2) is in line with a report by Leonard and Rosenberg in abstract form [17] on the effect of propylthiouracil on the 5'-deiodination of T$_4$ in rat kidney tissue preparations. It is, however, in conflict with a previous communication from this laboratory [11], where it was noted that the degree of inhibition by propylthiouracil of the deiodination of several iodothyronines in rat liver homogenate was similar whether or not incubations were carried out in the presence of exogenous cofactor (dithiothreitol). The reason for this apparent contradiction remains unclear but, obviously, the degree of inhibition by thiouracil derivatives will depend on the reaction conditions such as the

type and concentration of cofactor and the concentration of substrate.

The effects of thiouracil on the kinetic parameters of the 5'-deiodination of rT_3 (Fig. 1) are similar to the effects of a decrease in the concentration of dithiothreitol (Fig. 3), i.e. a proportional decrease in K_m and V.

It is concluded that conversion of rT_3 into $3,3'\text{-}T_2$ follows a ping-pong mechanism involving the formation of an intermediate which is reduced by dithiothreitol to the native enzyme. The intermediate may also react with thiouracil yielding an inactive complex. The importance of the sulfur in thiouracil is illustrated by the finding that uracil is devoid of inhibitory activity. It has been suggested that, beside the well-documented reaction of thiourea and thiouracil with sulfenyl iodides [4,6], thiourea may also react with -S-S-groups [3]. However, it has been shown that the reaction rate of thiouracil with disulfides is negligible compared with its reactivity towards sulfenyl iodides [4,6]. Reaction of thiouracil with sulfenyl iodides is much faster than the reaction of thiols with -SI groups [4]. In our study a significant effect of 0.5 μM thiouracil was observed in the presence of over 1 mM dithiothreitol. Therefore, our results strongly suggest that an enzyme sulfenyl (E-S$^+$) group — probably a sulfenyl iodide — is formed during 5'-deiodination of iodothyronines.

Because of the similarity in enzymatic 5- and 5'-deiodination — both are stimulated by mercapto compounds and inhibited by thiouracil derivatives — a general reaction pathway is proposed (Eqns. 2 and 3).

$$TI_n + \text{E-SH} \rightarrow TI_n \cdot \text{E-SH} \rightarrow \rightarrow \rightarrow \text{E-SI} + TI_{n-1} \qquad (2)$$

$$\text{E-SI} + 2 \text{ R-SH} \rightarrow \text{E-SH} + \text{R-S-S-R} + \text{HI} \qquad (3)$$
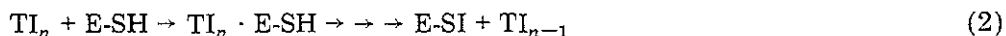
The formation of a non-covalent, iodothyronine-enzyme complex ($TI_n \cdot \text{E-SH}$) is followed by a displacement reaction, which may include generation of one or more distinct intermediates. Monodeiodinated iodothyronine (TI_{n-1}) is released and an enzyme-sulfenyl iodide (E-SI) is formed (Eqn. 2). The latter is reduced to the native state by cofactor (Eqn. 3). E-SI may also react with thiouracil (X-SH) yielding a mixed disulfide (E-S-S-X) (Eqn. 4).

$$\text{E-SI} + \text{X-SH} \rightarrow \text{E-S-S-X} + \text{HI} \qquad (4)$$

In both latter reactions the second product (I^-) is released. Free enzyme is obtained from E-S-S-X by reaction with cofactor [6] (Eqn. 5).

$$\text{E-S-S-X} + 2 \text{ R-SH} \rightarrow \text{E-SH} + \text{R-S-S-R} + \text{X-SH} \qquad (5)$$
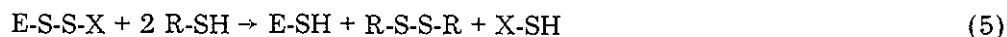
It should be emphasized that reactions 3 and 5 involve two thiol groups of the cofactor. In our case these are supplied by one molecule dithiothreitol.

A corollary of the present study is that the substrate is not directly reduced by the cofactor. Rather, the catalytic process is characterized by two reactions: transiodination (Eqn. 2) and subsequent reduction of the iodo-enzyme complex by cofactor (Eqn. 3).

Reminiscent of the work of Hartman et al. on non-enzymatic deiodination of di-iodotyrosine by cysteine [18—20], enzymatic 5'-deiodination may proceed by addition of a proton to $C^{5'}$ yielding (1). This is facilitated by the electron-donating effect of a dissociated phenolic hydroxyl group. An enzymic

sulfhydryl group may then assist the elimination of I⁺ by forming a sulfenyl iodide. This model, however, does not account for the finding that rT_3 is a much better substrate for the 5'-deiodinase than T_4, the K_m being 40-fold lower and the V being 20-fold higher at pH 7.2 and 37°C [12].

Another possible mechanism of action of iodothyronine-5'-deiodinase is related to that of thymidylate synthetase in the dehalogenation of 5-bromo- and 5-iodo-2'-deoxyuridylate (IdUMP) (Ref. 21, see also Refs. 10, 11). This implies a primary attack of an enzymic sulfhydryl group to $C^{6'}$ yielding a covalent enzyme-5'-6'-dihydrosubstrate complex (2). The elimination of I⁺ may
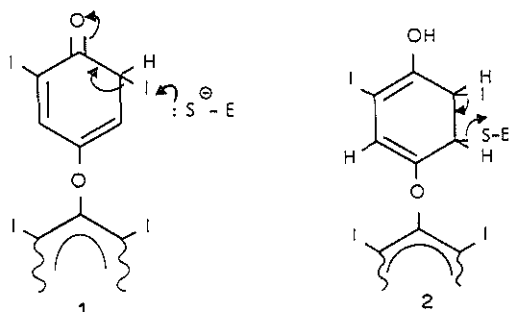
then be accomplished in a concerted mechanism as shown or with the aid of a second sulfhydryl group yielding an E-SI complex. Steric hindrance by bulky substituents on both C^3 and C^5 in T_4 may interfere with the approach of the enzymic sulfhydryl group to $C^{6'}$. Release of steric hindrance by deletion of one of these iodine atoms on the tyrosyl ring may account for the preferred reaction with rT_3. Also one can envisage 5-deiodination to occur by this mechanism and not by the former because of the absence of the activating hydroxyl group on the tyrosyl ring.

Because of the similarities between the structures of IdUMP, iodothyronines and 5-n-propyl-2-thiouracil — another strong inhibitor of the deiodination of thyroxine [2,22] — it was previously hypothesized [10,11] that the latter might inhibit the conversions competitively with respect to substrate. This appears to be ruled out by the present study.

Acknowledgements

References

1 Taurog, A. (1978) in: The Thyroid (Werner, S.C. and Ingbar, S.H., eds.), 4th edn., pp. 31—61, Harper and Row, New York
2 Hershman, J.M. and Van Middlesworth, L. (1962) Endocrinology 71, 94—100
3 Maloof, F. and Soodak, M. (1963) Pharmacol. Rev. 15, 43—95
4 Cunningham, L.W. (1964) Biochemistry 3, 1629—1634
5 Fawcett, D.M. (1968) Can. J. Biochem. 46, 1433—1441
6 Jirousek, L. (1968) Biochim. Biophys. Acta 170, 152—159
7 Jirousek, L. and Cunningham, L.W. (1968) Biochim. Biophys. Acta 170, 160—171
8 Visser, T.J., van der Does-Tobé, I., Docter, R. and Hennemann, G. (1976) Biochem. J. 157, 479—482

9 Chopra, I.J. (1977) Endocrinology 101, 453—463
10 Visser, T.J. (1978) Molec. Cell. Endocrinol. 10, 241—247
11 Visser, T.J., Fekkes, D., Docter, R. and Hennemann, G. (1978) Biochem. J. 174, 221—229
12 Visser, T.J., Fekkes, D., Docter, R. and Hennemann, G. (1979) Biochem. J. 179, 489—495
13 Visser, T.J., Krieger-Quist, L.M., Docter, R. and Hennemann, G. (1978) J. Endocrinol. 79, 357—362
14 Chopra, I.J., Wu, S.Y., Nakamura, Y. and Solomon, D.H. (1978) Endocrinology 102, 1099—1106
15 Hüfner, M. and Grussendorf, M. (1978) Clin. Chim. Acta 85, 243—251
16 Laidler, K.J. and Bunting, P.S. (1973) The Chemical Kinetics of Enzyme Action, Clarenden Press, Oxford
17 Leonard, J.L. and Rosenberg, I.N. (1977) Endocrinology 100, T-14
18 Hartmann, K., Hartmann, N. and Bulka, E. (1971) Z. Chem. 11, 344—345
19 Hartmann, K., Hartmann, N. and Bulka, E. (1971) Z. Chem. 11, 424—425
20 Friedman, M. (1973) The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins, pp. 170—172, Pergamon Press, Oxford
21 Wataya, Y. and Santi, D.V. (1975) Biochem. Biophys. Res. Commun. 67, 818—823
22 Visser, T.J., van der Does-Tobé, I., Docter, R. and Hennemann, G. (1975) Biochem. J. 150, 489—493

BBA 68912

MECHANISM OF INHIBITION OF IODOTHYRONINE-5'-DEIODINASE BY THIOUREYLENES AND SULFITE

THEO J. VISSER

Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty, Erasmus University, Rotterdam (The Netherlands)

Summary

Previous studies have demonstrated that thiouracil inhibits the 5'-deiodination of 3,3',5'-triiodothyronine uncompetitively with respect to substrate and competitively with respect to cofactor (thiol compounds). This paper shows that sulfite is also a strong inhibitor of this reaction showing a dose-dependent effect between 1 μM and 1 mM. The mode of inhibition is similar to that described for thiouracil. Dose-dependent inhibition was also observed with thiosulfate (0.01—1 mM), iodide and thiocyanate (both greater than 1 mM). No effect was exerted by up to 10 mM cyanide and up to 100 mM azide. Methimazole and thiourea were weak inhibitors above 0.1 mM but inhibition did not reach completion. These experiments were carried out in the presence of 1 mM dithiothreitol. The effect of thiouracil was found to be competitively obviated by methimazole and thiourea. However, the effeft of sulfite and that of methimazole or thiourea were additive. It is proposed that an enzyme-sulfenyl iodide is formed during deiodination (ping-pong mechanism). This sulfenyl iodide may be reduced by cofactor to yield native enzyme. It may also react with thioureylenes, yielding mixed disulfides, or with sulfite, yielding a thiosulfate. The enzyme-methimazole disulfide is apparently less stable than the enzyme-thiouracil complex. It is suggested that sulfite also reacts with the enzyme-thioureylene disulfide.

Introduction

2-Thiouracil (1) derivatives and 2-mercapto-1-methylimidazole (methimazole; 2) are used in the treatment of hyperthyroidism.
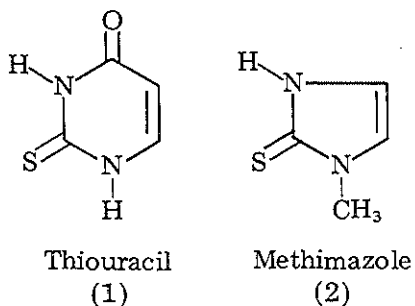
Scheme I. Structures of thiouracil and methimazole.

These drugs interfere with the biosynthesis of thyroxine by inhibiting thyroid peroxidase activity [1]. Of these compounds only the thiouracil derivatives have an additional effect on the deiodination of thyroid hormone in peripheral tissues [1]. It has been shown that thiouracil derivatives uncompetitively inhibit the 5'-deiodination of thyroxine, yielding 3,3'5-triiodothyronine (triiodothyronine) [2], and of 3,3',5'-triiodothyronine (reverse triiodothyronine), yielding 3,3'-diiodothyronine (diiodothyronine) [3,4]. Thyroid hormone-deiodinating enzymes contain essential sulfhydryl groups and thiol compounds are required for activity [5]. Thiouracil has been shown to react selectively with sulfenyl iodides forming mixed disulfides [6]. It was, therefore, proposed that deiodination follows a ping-pong mechanism implying the intermediate formation of an iodo-enzyme complex where the essential -SH group is converted into a -SI group [4]. In the uninhibited reaction this E-SI complex is subsequently reduced by cofactor [4]. The present study was undertaken to test this hypothesis by investigating the effects on the 5'-deiodination of reverse triiodothyronine by agents such as sulfite known to react with a sulfenyl sulfur. In addition, the interaction of methimazole and thiourea with the enzyme inhibited by thiouracil or sulfite was studied. The results demonstrate that the mode of inhibition by sulfite is similar to that previously shown for thiouracil [4]. However, inhibition by thiouracil is competitively obviated by methimazole and thiourea, whereas the effects of the latter compounds and that of sulfite are additive.

Methods

The conversion of reverse triiodothyronine into diiodothyronine by rat liver microsomal fraction in the presence of dithiothreitol was measured essentially as described previously [7]. In short, usually 0.1 μM reverse triiodothyronine was incubated in 0.25 ml 0.05 M phosphate containing 3 mM EDTA, 1 mM dithiothreitol (unless indicated otherwise) and other substances to be tested (pH 6.5) with 7 μg of microsomal protein/ml for 20 min at 37°C. The reaction was stopped by the addition of 1 ml 0.06 M barbitone buffer containing 0.1% bovine serum albumin and 0.1% SDS (pH 8.6). The amount of diiodothyronine produced was measured with a specific radioimmunoassay in 50 μl of the extract [8]. The reaction was started by the addition of microsomes.

Deiodinase activity was corrected for non-enzymic production of diiodo-thyronine as measured in extracts of control incubations. In the controls, microsomes were added after the barbitone-SDS buffer. Usually, the amount of diiodothyronine produced in the absence of enzyme was negligible (less than 5%) compared with that generated enzymically. Incubation and radioimmuno-assay were performed in duplicate.

Results

At the concentration of microsomes used there is no significant binding of reverse triiodothyronine to non-enzymic constituents of this fraction and degradation of diiodothyronine is negligible [7]. Alterations in diiodothyronine accumulation by test substances either via an effect on substrate availability or via an effect on the stability of the product are, therefore, excluded. A dose-dependent inhibition of diiodothyronine production was observed with 0.01—10 μM thiouracil, 1 μM—1 mM sulfite, 0.01—1 mM thiosulfate and 1—100 mM iodide or thiocyanate (Fig. 1). Deiodinase activity was also lowered by methimazole and thiourea at concentrations above 0.1 mM. Inhibition by these compounds reached a plateau of only approx. 50% despite increasing their concentration above 10 mM. No effect was observed with up to 10 mM cyanide and up to 100 mM azide. A 50% reduction in deiodinase activity was obtained with 0.3 μM thiouracil, 0.02 mM sulfite, 0.2 mM thiosulfate and 20 mM thiourea, iodide or thiocyanate but not with even higher concentrations of methimazole.

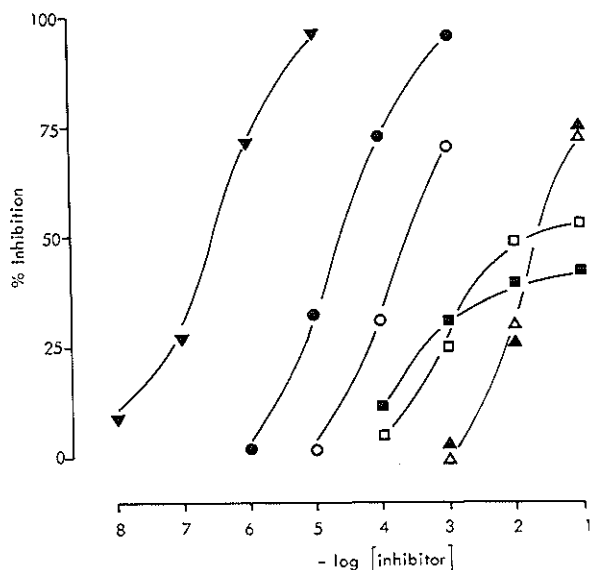The effect of addition of 0.04 or 0.1 mM sulfite on the kinetics of the reac-

Fig. 1. Inhibition of the conversion of reverse triiodothyronine into 3,3'-diiodothyronine by various compounds. The following substances were tested: ▼, thiouracil (TU); ●, SO_3^{2-}; ○, $S_2O_3^{2-}$; ▲, I^-; △, SCN^-; □, thiourea, and ■, methimazole (MMI). For details see Methods. Results are means of 3—8 closely agreeing experiments.
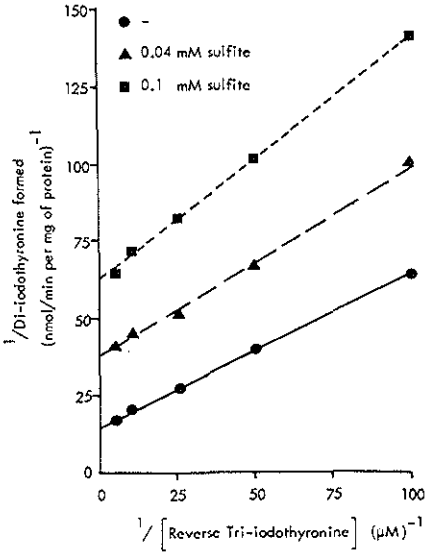
Fig. 2. Lineweaver-Burk plot of 3,3'-diiodothyronine production rate as a function of reverse triiodo-thyronine concentration and the effect of SO_3^{2-}. The following concentrations of SO_3^{2-} were tested: 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of three closely agreeing experiments.
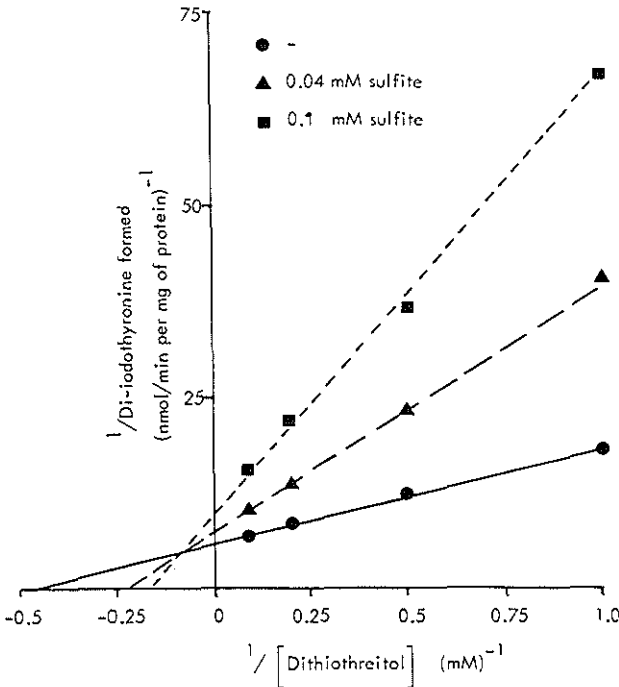

Fig. 3. Lineweaver-Burk plot of 3,3'-diiodothyronine production rate as a function of dithiothreitol concentration and the effect of SO_3^{2-}. The following concentrations of SO_3^{2-} were tested: 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of two closely agreeing experiments.

tion was investigated. This resulted in virtually parallel displacements of the Lineweaver-Burk plot of the deiodination rate versus reverse triiodothyronine concentration (Fig. 2). This indicates that inhibition by sulfite is largely uncompetitive with respect to substrate. Increasing the concentration of dithiothreitol alleviated the effect of sulfite although not completely. Analysis by means of a double-reciprocal plot of diiodothyronine production rate versus dithiothreitol concentration revealed that the reaction of sulfite with the enzyme was largely competitive with cofactor (Fig. 3). These conclusions were supported by Dixon plots [9] of Figs. 2 and 3. In addition, it was found that the replots [9] of the $1/v$ axis intercepts in Fig. 2 and of the slopes in Fig. 3 as a function of SO_3^{2-} concentration were linear.

In the experiments shown in Fig. 4 addition of 0.4 or 1 μM thiouracil alone resulted in a suppression of deiodinase activity by 52 and 70%, respectively. However, inhibition declined to 44 and 44%, respectively, in the presence of increasing concentrations of methimazole. This is virtually the maximum level of inhibition obtained with methimazole alone, i.e. 38%. The effects of thiouracil are, therefore, competitively obviated by methimazole.

Addition of 0.04 or 0.1 mM sulfite suppressed diiodothyronine production rate by 59 and 72%, respectively (Fig. 5). Now, in the presence of methimazole inhibition was further increased to a maximum of 79 and 87%, respectively. Inhibition by methimazole alone in this case was at the maximum 48%. The effects of sulfite appear, therefore, to be additive to that of methimazole. In the experiments described in Figs. 2—5 very similar findings were obtained by
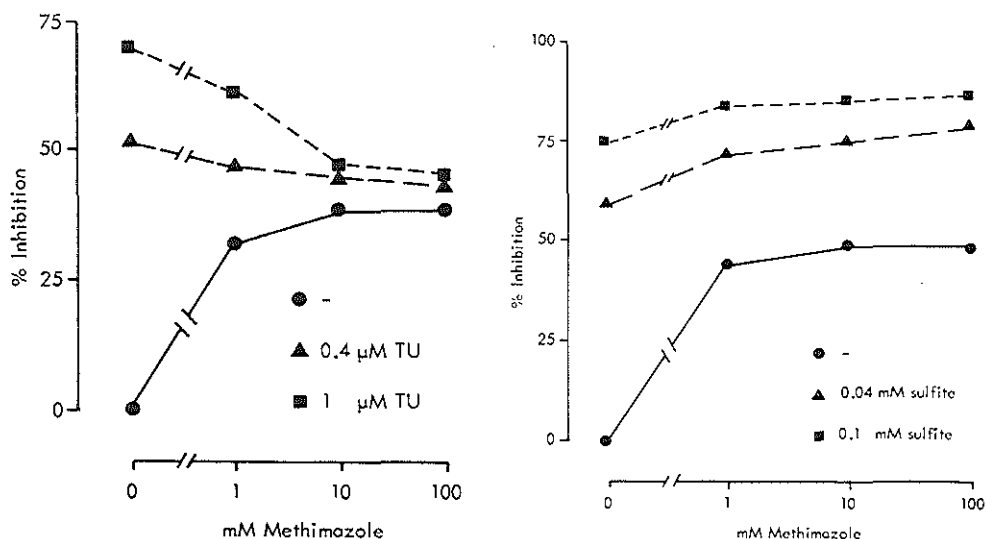

Fig. 4. Inhibition of the conversion of reverse triiodothyronine into 3,3'-diiodothyronine by the simultaneous addition of thiouracil (TU) and methimazole (MMI). TU was tested at concentrations of 0 (●), 0.4 (▲) and 1 (■) μM. For details see Methods. Results are means of four closely agreeing experiments.

Fig. 5. Inhibition of the conversion of reverse triiodothyronine into 3,3'-triiodothyronine by the simultaneous addition of SO_3^{2-} and methimazole (MMI). SO_3^{2-} was tested at concentrations of 0 (●), 0.04 (▲) and 0.1 (■) mM. For details see Methods. Results are means of two closely agreeing experiments.

using 0.4 and 1 mM thiosulfate instead of sulfite and 1—100 mM thiourea instead of methimazole.

It was found that the effects of the combined additions of thiouracul and sulfite, of thiouracil and iodide or of thiouracil and thiocyanate, tested in various proportions, were larger than the effects excerted by these compounds alone.

Discussion

When this study was in progress Leonard and Rosenberg [10] also reported on the effect of 6-propylthiouracil on the 5'-deiodination of thyroxine by rat kidney microsomal preparations. These investigators found that the inhibitory effect of thiouracil and propylthiouracil was attenuated by dithiothreitol, methimazole and thiourea but not by thiocyanate. They, however, noted a full restoration of deiodinase activity by 1 mM thiourea or methimazole, which in themselves did not inhibit triiodothyronine production. This discrepancy with our observation may be related to the differences in experimental conditions such as the choice of substrate and enzyme preparations, and the concentrations thereof, difference in dithiothreitol concentrations (0.1 mM [10] or 1 mM) and the absence [10] or presence (this paper) of air oxygen. In essence, nevertheless, their findings are in agreement with those presented by us previously [4] and in the present paper.

It has been demonstrated by Leonard and Rosenberg [10] and by us [4] that enzymic 5'-deiodination follows a ping-pong mechanism. Reaction of the first substrate (iodothyronine) with the enzyme results in the formation of an intermediate enzyme-complex, which by reaction with the second substrate (thiol cofactor) is converted back into native enzyme. Since it has been shown [5] that iodothyronine-deiodinating enzymes contain essential cysteine residues it is quite conceivable that during deiodination a sulfhydryl group is being oxidized. In consideration of the high reactivity of thiouracil towards sulfenyl iodides compared with ordinary disulfides [6] the formation of an E-SI complex in the catalytic cycle was implied [4]. The findings that thiouracil inhibits this reaction uncompetitively with substrate [2—4,10] and competitively with cofactor [4,10] support this hypothesis. They demonstrate that thiouracil reacts only with an intermediate in the deiodination process, being also the site of reaction with cofactor (see Fig. 6). It is not excluded, however, that inhibition by thiouracil is due to a reaction with some other form of sulfenyl sulfur such as an activated (protonated) disulfide [11—15]. To test this possibility the effect of several agents known to react with protein disulfides was investigated.

An intriguing observation was the high reactivity of SO_3^{2-} in contrast to the inactivity of CN^-. Cyanide is at least as reactive as SO_3^{2-} towards both disulfides [11—13] and sulfenyl iodides [6]. It should, however, be kept in mind that the present experiments were carried out in the presence of 1 mM dithiothreitol. The products of the reaction of SO_3^{2-} and CN^- with both a sulfenyl iodide and a disulfide are a thiosulfate and a thiocyanate, respectively. The difference in effect of sulfite and cyanide on the production of diiodothyronine may well be due to differences in the rate of regeneration of the

sulfhydryl group from these products by dithiothreitol. Iodide and thiocyanate were found to be weak inhibitors of 5′-deiodinase activity (Fig. 1).

Inhibition by sulfite is largely uncompetitive with substrate and competitive with cofactor. Thus, while belonging to an entirely different class of compounds, the mode of inhibition of the 5′-deiodination of thyroid hormone by SO_3^{2-} is similar to that by thiouracil. These findings provide further evidence for the formation of an enzyme-sulfenyl group (E-S$^+$) in this reaction.

Alleviation of the inhibitory activity of thiouracil by methimazole may be due either to competition of these compounds for the sulfenyl group in the intermediate enzyme complex [6] (Fig. 6, Reactions 2 and 3) or to reaction of methimazole with the enzyme-thiouracil mixed disulfide. The reaction rate especially of methimazole but also that of thiourea with β-lactoglobulin sulfenyl iodide are much higher compared with thiouracil [6]. This is in contrast with the results presented in this paper. It is shown that inhibition at saturating concentrations of methimazole is far from complete. This suggests that the mixed disulfide of enzyme with methimazole is rapidly reduced by cofactor (Fig. 6, Reaction 5). Recent studies on the structure-activity relationship of thioureylenes have shown that the low activity of methimazole is primarily due to the methylation of N_1 [16].

The mode of inhibition by SO_3^{2-} is very similar to that of thiouracil. The different behaviour of methimazole with the SO_3^{2-}-inhibited enzyme compared with its action in the presence of thiouracil was, therefore, unexpected. It has been reported that besides methimazole other compounds such as SO_3^{2-} react with β-lactoglobulin-thiouracil disulfide [17]. This suggests that inhibition by sulfite is not prevented by methimazole but that reaction with SO_3^{2-} may even be accelerated by prior formation of the enzyme mixed disulfide with methimazole (Fig. 6, Reactions 3 and 6). It should be noted that in the presence of sulfite there is minor irreversible loss of enzyme activity, which is not overcome by increasing dithiothreitol concentrations (Fig. 3). It is not excluded, therefore, that the effects observed with the simultaneous addition of SO_3^{2-} and methimazole may be accounted for to some extent by this action of sulfite.

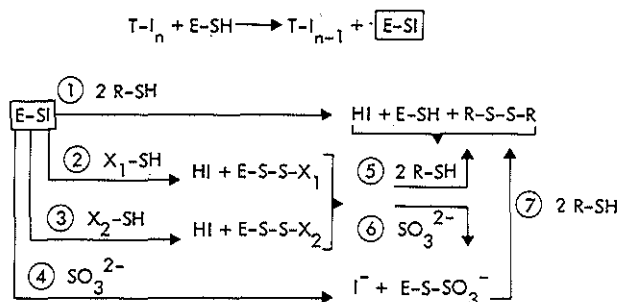Fig. 6 is shown in an attempt to clarify the several observations described in

Fig. 6. Possible interactions of thiouracil (X_1-SH), methimazole (X_2-SH) and SO_3^{2-} with iodothyronine 5′-deiodinase. Unaltered thiouracil and methimazole are released in reactions 5 and 6. Similarly, unaltered SO_3^{2-} is a product of Reaction 7. T-I_n, iodothyronine.

this paper. Recent studies in our laboratory are compatible with this view, since it has been found [18] that binding of radioiodinated propylthiouracil to rat liver microsomal fraction is induced specifically by substrates of the iodothyronine-5'-deiodinase. This binding is prevented competitively by dithiothreitol, unlabelled propylthiouracil, methimazole and sulfite. Since, apparently, Reaction 5 for the enzyme-thiouracil mixed disulfide and Reaction 7 are slow compared with Reaction 1 (Fig. 6), thiouracil and sulfite may be regarded as dead-end inhibitors. This is substantiated by the finding of linear replots of Figs. 2 and 3 [9].

In conclusion, the present study provides further evidence that an enzyme-sulfenyl group, probably a sulfenyl iodide, is formed during 5'-deiodination of iodothyronines. In view of the lability of -SI groups in aqueous media [6] it will, however, be a difficult task to prove the actual formation of such a derivative of the 5'-deiodinase.

Acknowledgements

References

1 Green, W.L. (1978) in The Thyroid (Werner, S.C. and Ingbar, S.H., eds.), 4th edn., pp. 77—87, Harper and Row, New York
2 Chopra, I.J. (1977) Endocrinology 101, 453—463
3 Chopra, I.J., Wu, S.Y., Nakamura, Y. and Solomon, D.H. (1978) Endocrinology 102, 1099—1106
4 Visser, T.J. (1979) Biochim. Biophys. Acta 569, 302—308
5 Visser, T.J. (1978) Mol. Cell. Endocrinol. 10, 241—247
6 Cunningham, L.W. (1964) Biochemistry 3, 1629—1634
7 Visser, T.J., Fekkes, D., Docter, R. and Hennemann, G. (1979) Biochem. J. 179, 489—495
8 Visser, T.J., Krieger Quist, L.M., Docter, R. and Hennemann, G. (1978) J. Endocrinol. 79, 357—362
9 Segel, I.H. (1975) Enzyme Kinetics, John Wiley and Sons, New York
10 Leonard, J.L. and Rosenberg, I.N. (1978) Endocrinology 103, 2137—2144
11 Parker, A.J. and Kharasch, N. (1959) Chem. Rev. 59, 583—628
12 Parker, A.J. and Kharasch, N. (1960) J. Am. Chem. Soc. 82, 3071—3075
13 Maloof, F. and Soodak, M. (1961) J. Biol. Chem. 236, 1689—1692
14 Maloof, F., Smith, S. and Soodak, M. (1969) Mechanisms of Reactions of Sulfur Compounds, Vol. 4, pp. 61—68.
15 Bäuerlein, E. and Keihl, R. (1976) FEBS Lett. 61, 68—71
16 Visser, T.J., van Overmeeren, E., Fekkes, D., Docter, R. and Hennemann, G. (1979) FEBS Lett. 103, 314—318
17 Jirousek, L. (1968) Biochim. Biophys. Acta 170, 152—159
18 Visser, T.J. and van Overmeeren, E. (1979) Biochem. J. 183, 167—169

SUBSTRATE REQUIREMENT FOR INACTIVATION OF IODOTHYRONINE 5'-DEIODINASE ACTIVITY
BY THIOURACIL

Theo J. Visser and Ellen van Overmeeren,
Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty,
Erasmus University, Rotterdam, The Netherlands.

SUMMARY

    Preincubation of rat liver microsomal fraction with 1 μM 2-thiouracil and
either 0.01-1 μM 3,3',5'-triiodothyronine, 0.01-1 μM 3',5'-diiodothyronine,
0.1-10 μM thyroxine or 0.1-10 μM 3,5-diiodothyronine led to a persistent,
progressive and concomitant decrease in subsequently assayed 3,3',5'-triiodo-
thyronine and 3',5'-diiodothyronine 5'-deiodinase activity. Preincubation with
thiouracil alone, with iodothyronines alone or with thiouracil and 10 μM
thyronine or 3,5-diiodotyrosine had virtually no effect. The results indicate
that (1) a previously proposed ping-pong mechanism for thyroid hormone deiodin-
ation, involving the formation of an enzyme-sulfenyl iodide intermediate, is
correct; (2) thyroxine, 3,3',5'-triiodothyronine and 3',5'-diiodothyronine are
substrates for a common 5'-deiodinase; (3) this 5'-deiodinase is not fully
specific as regards the position of the iodine substituents in the substrate,
since it also appears to catalyse the 5-deiodination of 3,5-diiodothyronine.

INTRODUCTION

    The main route of metabolism of the iodoamino acids, iodotyrosines and
iodothyronines, is by means of deiodination. In both instances the enzymatic
reaction is a reductive process, yet different types of enzymes are involved.
Deiodination of iodotyrosines is catalysed by a microsomal enzyme complex,
which uses reductive equivalents supplied by NADPH (Goswami and Rosenberg,
1977). Iodothyronine deiodinase activity is also located in the microsomal
fraction of several tissues, but present evidence indicates that it constitutes
a single enzyme, where reductive equivalents are supplied by thiols (Visser et
al, 1976; Fekkes et al, 1980).
    In the deiodination of 3,3',5,5'-tetraiodothyronine (thyroxine; T_4) two
types of reaction may be distinguished, i.e. deiodination of the phenolic ring
(5'-deiodination), yielding 3,3',5-triiodothyronine (T_3), and deiodination of
the tyrosyl ring (5-deiodination), yielding 3,3',5'-triiodothyronine (rT_3).

Both T_3 and rT_3 are subject to further degradation by both 5- and 5'-deiodination. Of all occurring iodothyronines T_3 is biologically the most active, suggesting that deiodination of T_4 is a possible site for the regulation of thyroid hormone activity at the level of peripheral tissues. It has been suggested (Visser, 1978) that 5- and 5'-deiodinations are mediated by separate enzymes (iodothyronine 5- and 5'-deiodinase). As yet no direct evidence has been presented to support this hypothesis. On the contrary, subcellular fractionation (Fekkes et al, 1979) and analysis of detergent extracts of microsomes with various techniques (Fekkes et al, 1980) have failed to separate 5- and 5'-deiodinase activity.

Recent findings (Leonard and Rosenberg, 1978, 1980; Visser, 1979, 1980) suggest that deiodination follows a ping-pong mechanism. This involves the transfer of an iodinium ion from the substrate to a sulfhydryl group of the enzyme leading to the formation of an enzyme-sulfenyl iodide (E-SI) complex. The E-SI intermediate is subsequently reduced by mercapto compounds (cofactor) to free enzyme. The enzyme is inhibited by derivatives of 2-thiouracil (TU) as these compounds react with the E-SI intermediate forming an enzyme-TU mixed disulfide (dead-end complex). This is supported by the finding that inhibition by TU is uncompetitive with substrate and competitive with cofactor. Thus, TU reacts with the enzyme only after formation of the E-SI intermediate and, therefore, only if substrate is present. This appears to be true as binding of radioactive TU to rat liver microsomal fraction is specifically induced by substrates of the 5'-deiodinase (Visser and Van Overmeeren, 1979; Leonard and Rosenberg, 1980). Moreover, the persistent inactivation of T_4 5'-deiodinase activity by TU requires the presence of T_4 (Leonard and Rosenberg, 1978, 1980).

Results from previous experiments have suggested that the 5'-deiodination of several iodothyronines is mediated by a single enzyme. Thus, rT_3 is a competitive inhibitor of the 5'-deiodination of T_4 and vice versa where apparent K_m and K_i values were found to be identical (Kaplan and Utiger, 1978; Visser et al, 1979a). Furthermore, the effect of pH on the 5'-deiodination of rT_3 and 3',5'-diiodothyronine (3',5'-T_2) was found to be very similar (Visser and Van Overmeeren, 1980). Based on the above reaction model the possibility of a single enzyme catalysing the 5'-deiodination of the different iodothyronines has now been tested more directly.

5'-Deiodinase activity of rat liver microsomal fraction was inactivated by coincubation with TU and rT_3, 3',5'-T_2, T_4 or, though to a lesser extent, also with 3,5-T_2 but not with 3,5-diiodotyrosine (DIT) or thyronine (T_0). These results indicate that T_4, rT_3 and 3',5'-T_2 are indeed substrates for a common

5'-deiodinase. The specificity of this enzyme, however, is not complete since it also appears to catalyse, though with lesser efficiency, the deiodination of $3,5-T_2$.

MATERIALS AND METHODS

Materials

L-Thyronine, 3,5- and 3',5'-diiodo-L-thyronine, 3,3',5'-triiodo-L-thyronine, L-thyroxine and 3,5-diiodo-L-tyrosine were of the highest purity available and were purchased from Henning Berlin GmbH, Germany. 2-Thiouracil, N-ethylmaleimide and D,L-dithiothreitol were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Methods

Rat liver microsomal fraction was prepared essentially as described (Visser et al, 1976). Aliquots of this preparation (final protein concentration 400 µg/ml) were incubated at 37^OC with 1 µM TU and various concentrations of iodoamino acids or T_0 in 0.05 M phosphate, 3 mM EDTA, 0.1 mM dithiothreitol (DTT), pH 6.5. After 30 min the reaction mixtures were diluted 10-fold by the addition of 0.05 M phosphate, 3 mM EDTA, pH 6.5, at 0° C. In control experiments, microsomes were incubated without iodoamino acids or T_0 which were added only after the dilution of the reaction mixtures. The resulting suspensions were kept at 0° C until the assay of 5'-deiodinase activity. For this, aliquots of the mixtures were incubated at 37° C with equal volumes of 0.05 M phosphate, 3 mM EDTA, 2 mM DTT, pH 6.5, containing 2 µM $3',5'-T_2$ or 0.05 µM rT_3. After 15 min the reaction was stopped by the addition of 9 volumes 0.06 M barbitone, 0.15 M NaCl, 0.1% bovine serum albumin, 0.1% sodium dodecyl sulfate, pH 8.6, at room temperature. In controls, substrate was added only after the sodium dodecyl sulfate-buffer. The products formed (3'-iodothyronine and $3,3'-T_2$, respectively) were measured in duplicate by specific radioimmunoassays in 50 µl of the extracts (Visser et al, 1979a; Visser and Van Overmeeren, 1980).

In a similar type of experiment microsomes were preincubated for 30 min at 37° C with 1 mM N-ethylmaleimide (NEM) and 0.1-1 µM $3',5'-T_2$. The reaction mixtures were now diluted with 0.05 M phosphate, 3 mM EDTA, 2 mM DTT, pH 6.5, to block unreacted NEM. The subsequent assay of 5'-deiodinase activity with $3',5'-T_2$ as the substrate was performed as described above.

RESULTS

Preincubation of rat liver microsomal fraction with thiouracil alone or with iodothyronines alone did not affect the 5'-deiodination of rT_3 or $3',5'-T_2$ in the second incubation. However, coincubation of microsomes with 1 μM TU and either 0.01-1 μM rT_3, 0.01-1 μM $3',5'-T_2$, 0.1-10 μM T_4 or 0.1-10 μM $3,5-T_2$ resulted in a persistent and progressive loss of rT_3 5'-deiodinase activity (Fig. 1). After incubation of microsomes with TU in the presence of 10 μM T_0 or DIT, rT_3 5'-deiodinase activity was found to be 91 ± 9% and 80 ± 7% (mean ± S.D., n=6) of the control value, respectively. The presence of as little as 0.01 μM rT_3 or $3',5'-T_2$ in the preincubation resulted in a loss of 60% of enzyme activity in both cases. The addition of 0.1 μM T_4 or 1 μM $3,5-T_2$ to the reaction mixtures during the preincubation led to an persistent decrease in 5'-deiodinase activity by 40% and 60%, respectively (Fig. 1.). Under the conditions tested, maximum inhibition was obtained with 0.1 μM rT_3 or $3',5'-T_2$, 1 μM T_4 and 10 μM $3,5-T_2$ and amounted to approximately 80%.
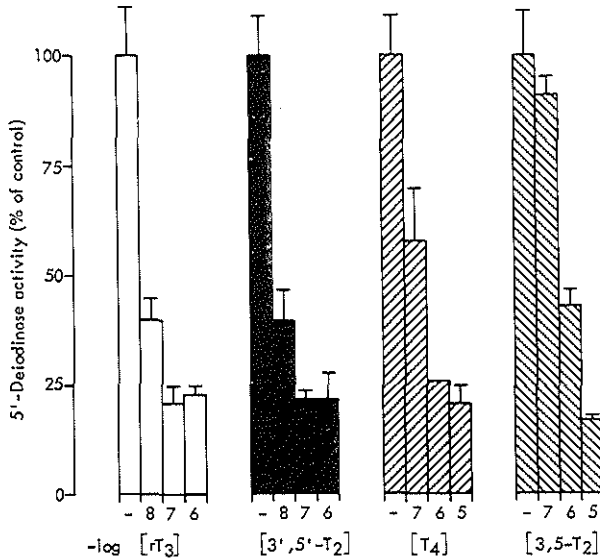

Fig. 1. Effect of preincubation of rat liver microsomal fraction with 1 μM TU with or without various concentrations of rT_3, $3',5'-T_2$, T_4 or $3,5-T_2$ on the subsequent assay of rT_3 5'-deiodinase activity. For details see text. Results are expressed as percentage of control and are given as mean ± S.D. (n=4-8).

Figure 2 shows that coincubation of microsomes with 1 μM TU and increasing concentrations of $3',5'-T_2$, T_4 or $3,5-T_2$ led to a progressive, parallel loss of rT_3 and $3',5'-T_2$ 5'-deiodinase activity. Irrespective of the substrate used in the second incubation, virtually maximum(approximately 70%) inhibition was reached again using 0.1 μM rT_3 or $3',5'-T_2$, 1 μM T_4 or 10 μM $3,5-T_2$.

Even after the subsequent addition of excess DTT, $3',5'-T_2$ 5'-deiodinase activity was found to be greatly inhibited following the reaction of microsomes with 1 mM NEM. Compared with the control experiment, where NEM was added only after the DTT, only 4-5% of deiodinase activity was left. The presence of 0.1 or 1 μM $3',5'-T_2$ during the preincubation did not protect against the inhibitory activity of NEM. This resulted only in an increase in the subsequent 5'-deiodination of $3',5'-T_2$ to 7-8% of the control.
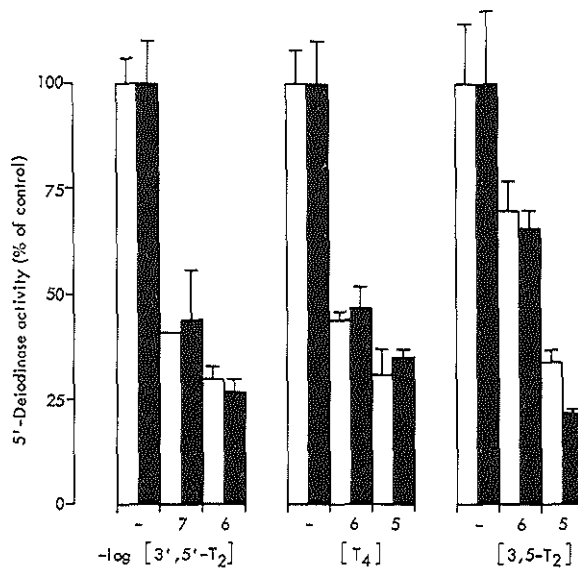

Fig. 2. Effect of preincubation of rat liver microsomal fraction with 1 μM TU with or without various concentrations of $3',5'-T_2$, T_4 or $3,5-T_2$ on the subsequent assay of rT_3 (□) and $3',5'-T_2$ (■) 5'-deiodinase activity. For details see text. Results are expressed as percentage of control and are given as mean ± S.D. (n=4-8).

DISCUSSION

    The conditions for the experiments described in the previous section were
chosen such that concentrations of TU and iodothyronines were high enough in
the first incubation to ensure a proper interaction with the enzyme. After
dilution, the concentrations of these compounds would need to be decreased as
much as to prevent the interference with the subsequent assay of deiodinase
activity. Previous studies had shown that 0.1 and 1 μM TU inhibit enzyme activ-
ity by 25 and 75%, respectively, in reaction with mixtures containing 0.1 μM
rT_3 (substrate) and 1 mM DTT (cofactor) at pH 6.5 (Visser et al, 1979b). In
the present experiments, the mixtures contained 1 μM TU and 0.1 mM DTT in the
first, and 0.05 μM TU and 1 mM DTT in the second incubation. At pH 6.5 in the
presence of 3 mM DTT, apparent K_m values for T_4, rT_3 and $3',5'-T_2$ are 3, 0.04
and approximately 0.1 μM, respectively. Deiodination rates of rT_3 and $3',5'-T_2$
are also much higher compared with T_4 (Visser et al, 1979a; Visser and Van Over-
meeren, 1980; D. Fekkes, E. van Overmeeren and T.J. Visser, unpublished work).
The concentrations of $3',5'-T_2$ and T_4 were at the most 1, 1 and 10 μM in the
first, and 0.05, 0.05 and 0.5 μM, respectively, in the second incubation. In
the presence of saturating concentrations of substrate in the second incubation,
the small amount of iodothyronine carried over from the first incubation would,
therefore, not interfere with the estimation of enzyme activity. These consider-
ations were borne out by the findings that enzyme activity measured after in-
cubation of microsomes with TU alone or with iodothyronine alone was not differ-
ent from the control experiment.

    It has been shown previously that only in the presence of substrate the
reaction of TU with the 5'-deiodinase leads to a persistent inactivation of the
enzyme (Leonard and Rosenberg, 1978; 1980; Visser, 1979). This is probably the
result of the formation of an enzyme-TU mixed disulfide by reaction of TU with ·
an enzyme-sulfenyl iodide (E-SI) intermediate. We now have demonstrated that
preincubation of rat liver microsomal fraction with TU and low concentrations
of rT_3, $3',5'-T_2$ or T_4 results in a decrease in the subsequently assayed 5'-
deiodinase activity. These findings not only confirm the proposed mechanism
of inhibition by TU, but also prove that indeed these iodothyronines are sub-
strates for a common 5'-deiodinase. This is substantiated once more by the
observation that the effects on 5'-deiodinations of both rT_3 and $3',5'-T_2$ were
very similar depending on the conditions during the preincubation. The specific-
ity of the inactivation process is illustrated by the finding that the inhibit-
ory activity of TU is not or virtually not expressed in the presence of high

concentrations of T_0 or DIT. Moreover, the relative activity of T_4, rT_3 and $3',5'-T_2$ in the induction of dead-end complex formation by TU mirrors their performance as substrates for the 5'-deiodinase. This is further support for the concept that compounds which assist in the inactivation of the 5'-deiodinase by TU are also substrates for this enzyme.

Thus, there appears to be an absolute requirement for the presence of substrate in the persistent inactivation of the 5'-deiodinase by TU. Unexpectedly, $3,5-T_2$ also fulfilled this requirement, despite the absence of iodine substituents in the 3' and 5' positions. These results lead to the inevitable conclusion that $3,5-T_2$ is also deiodinated by the same enzyme which mediates the 5'-deiodination of other iodothyronines. Both 5- and 5'-deiodinations may, therefore, be catalysed by the same enzyme. Whether this reflects that only a single enzyme is involved in the entire sequential deiodination of thyroxine remains to be investigated. Published observations of the deiodination of thyroid hormone in vivo and in vitro are best explained by the two-enzyme hypothesis. If this hypothesis proves to be correct our results indicate that these enzymes have no absolute specificity. Recent studies have also shown that at high concentrations $3,5-T_2$ is a competitive inhibitor of the 5'-deiodination of $3',5'-T_2$ (D. Fekkes, E. van Overmeeren and T.J. Visser, unpublished work).

The experiments involving NEM were also intended to give information on the substrate specificity of the 5'-deiodinase. If the enzyme-sulfhydryl group actively involved in the deiodination process, were the only one exposed to the environment, one would anticipate that occupation of the active site with substrate would prevent NEM from blocking this group. In that case it would be possible to test by an entirely different approach which iodothyronines are substrates for a common enzyme. The results once again demonstrate that thyroid hormone-deiodinating enzymes contain one or more essential cysteine residues. The failure of $3',5'-T_2$ to protect against the inactivation by NEM may suggest that the catalytic thiol group is not shielded sufficiently by the substrate under the conditions tested. It may also indicate that essential sulfhydryl groups are also located outside the active site of the enzyme.

In conclusion, the present results demonstrate that $3,5-T_2$ is deiodinated by the enzyme catalysing the 5'-deiodination of other iodothyronines. The results at least indicate that thyroid hormone deiodinating enzymes do not display full specificity with respect to the position of the iodine atoms in the substrate.

REFERENCES

Fekkes, D., van Overmeeren-Kaptein, E., Docter, R., Hennemann, G. & Visser, T.J. (1979) Biochim. Biophys. Acta 587, 12-19.

Fekkes, D., van Overmeeren, E., Hennemann, G. & Visser, T.J. (1980) Biochim. Biophys. Acta 613, 41-51.

Goswami, A. & Rosenberg, I.N. (1977) Endocrinology 101, 331-341.

Kaplan, M.M. & Utiger, R.D. (1978) Endocrinology 103, 156-161.

Leonard, J.L. & Rosenberg, I.N. (1978) Endocrinology 103, 2137-2144.

Leonard, J.L. & Rosenberg, I.N. (1980) Endocrinology 106, 444-451.

Visser, T.J. (1978) Molec. Cell. Endocrinol. 10, 241-247.

Visser, T.J. (1979) Biochim. Biophys. Acta 569, 302-308.

Visser, T.J. (1980) Biochim. Biophys. Acta 611, 371-378.

Visser, T.J. & van Overmeeren, E. (1979) Biochem. J. 183, 167-169.

Visser, T.J. & van Overmeeren, E. (1980) Biochim. Biophys. Acta 631, 246-252.

Visser, T.J., van der Does-Tobé, I., Docter, R. & Hennemann, G. (1976). Biochem. J. 157, 479-482.

Visser, T.J., Fekkes, D., Docter, R. & Hennemann, G. (1979a) Biochem. J. 179, 489-495.

Visser, T.J., van Overmeeren, E., Fekkes, D., Docter, R. & Hennemann, G. (1979b). FEBS Lett. 103, 314-318.