pH-dependency of iodothyronine metabolism in isolated perfused rat liver

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1. Isolated livers from fed male rats were perfused for 2 h with T_4 (L-thyroxine), T_3 (L-3,3',5-tri-iodothyronine) or rT_3 (L-3,3',5'-tri-iodothyronine) at different pH values (7.1–7.6) in a fully synthetic medium, whereby normal metabolic functions were maintained without addition of rat blood constituents or albumin. 2. T_3 output into the medium and net T_3 production reached a maximum at a pH of the medium of 7.2 and significantly decreased with alteration of the pH when livers were perfused with T_4 as a substrate. 3. However, the net T_4 and T_3 uptake by the liver, as well as the hepatic T_4 and T_3 content after perfusion, were not dependent on the pH of the perfusion when livers were offered T_4 or T_3 as substrates respectively. 4. Determination of intracellular pH by the analysis of the distribution of the weak acid dimethylloxazolidinedione allows the conclusion that the pH optimum of iodothyronine 5'-deiodinase in the intact perfused liver corresponds to the maximum determined *in vitro* for the membrane-bound enzyme localized in the endoplasmic reticulum. 5. The rapid 5'-deiodination of rT_3 to 3,3'- T_2 (L-3,3'-di-iodothyronine), the fast disappearance of 3,3'- T_2 , and the fact that no net rT_3 production from T_4 could be detected, supports the hypothesis that in rat liver iodothyronine 5'-deiodinase activity seems to predominate over iodothyronine 5-deiodinase activity. 6. Thus the rat liver can be considered in normal physiological situations as an organ forming T_3 from T_4 and deiodinating rT_3 originating from extrahepatic tissues, whereby the cellular iodothyronine 5'-deiodination rate is controlled by the intracellular pH.

Extrathyroidal enzymic deiodination of the thyroid hormone L-thyroxine (T_4) to other iodothyronines results in both activation and inactivation of T_4 . One product, L-3,3',5-tri-iodothyronine (T_3) represents a compound that is more active, in terms of metabolic action and effects, than T_4 , whereas the other product, L-3,3',5'-tri-iodothyronine (rT_3) is regarded as calorigenically inactive (Jorgensen, 1978). The (two) enzyme(s) iodothyronine 5'-deiodinase and 5-deiodinase forming these different tri-iodothyronines exhibit different pH optima in rat liver microsomal fractions and liver homogenates *in vitro* [for a review, see Köhrle (1981)]. Uptake, deiodination and conjugation of iodothyronines by isolated perfused rat liver was initially detected by paper chromatography of the radiolabelled compounds (Briggs *et al.*, 1953; Flock

& Owen, 1965; Hillier, 1972). After introduction of specific radioimmunoassays, hepatic T_3 production from T_4 was found by Hesch *et al.* (1975) and by Jennings *et al.* (1979; Jennings, 1981), but not by van der Heide (1980). From these experiments the enzymic character of 5'-deiodination of T_4 in perfused rat liver became evident. The reaction was shown to be sensitive to temperature, linear with time, specifically inhibited by compounds such as 6-n-propyl-2-thiouracil or 3,5-di-iodo-4-hydroxybenzoic acid butyl ester, substrate-specific, saturable, dependent on concentration of the substrate T_4 (either the free T_4 in the medium or the uptake-mediated liver content of T_4). In addition, iodothyronine 5'-deiodinase activity itself is decreased in streptozotocin-induced diabetes in rats (Jennings, 1981). It has been suggested by Höffken *et al.* (1978) that changes in cellular pH under various physiological and pathophysiological conditions may contribute to the regulation of the iodothyronine deiodinative metabolism beside other regulatory factors [for a review, see Werner (1981)].

Abbreviations used: T_4 , L-thyroxine, L-3,3',5,5'-tetra-iodothyronine; T_3 , L-3,3',5-tri-iodothyronine; rT_3 , L-3,3',5'-tri-iodothyronine; 3,3'- T_2 , L-3,3'-di-iodothyronine; 3',5'- T_2 , L-3',5'-di-iodothyronine.

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The present study was therefore undertaken to test, by using the isolated perfused rat liver, the influence of intracellular pH on iodothyronine metabolism.

Materials and methods

Iodothyronines of highest purity were obtained through the generosity of Henning Berlin G.m.b.H. Reagents for T_4 radioimmunoassay were purchased from IMA (Gießen, Germany); L -[3'- 125 I] T_3 ($>1200 \mu\text{Ci}/\mu\text{g}$) and L -[125 I] rT_3 ($>1200 \mu\text{Ci}/\mu\text{g}$) were from Amersham-Buchler (Braunschweig, Germany). Other reagents were of highest purity commercially available.

Livers from fed male Wistar rats (Versuchstierzucht-Institut, Hannover, Germany) weighing about 10 g were isolated and perfused as described by Schimassek (1963), with a 10% (v/v) emulsion of Fluorocarbon 43 made up in the non-ionic detergent Pluronic F 68 by sonication and diluted with saline buffer (Krone *et al.*, 1974). By using this fully synthetic medium it was possible to perfuse without any further additions (e.g. erythrocytes, serum or albumin). The functional state of the organ was controlled by continuous measurement of flow (3–4 ml/min per g), pH, pO_2 and enzyme release {glutamic-oxaloacetic transaminase; L -aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1, [<1.5 units ($\mu\text{mol}/\text{min}$)/120 min per g]} into the perfusion medium. Determination of the metabolic function and of metabolites was as previously described (Krone *et al.*, 1974; Seitz *et al.*, 1977; Müller & Seitz, 1980). The following pH-dependent changes were observed (results are given as nmol/min per g $\pm$ S.D. for pH 7.38, and percentage of change at pH 7.20 and 7.56 for four livers in each case). Oxygen consumption: 1230 ± 40 ; -5% , $+14\%$; output of glucose (52 ± 18 ; $+329\%$, -460%), lactate (57 ± 24 ; -72% , $+196\%$), urea (86 ± 24 ; $+33\%$, $+17\%$), ketone bodies (94 ± 8 ; $+11\%$, -37%). These changes agree well with already published data (Seglen, 1972; Lueck & Miller, 1970; Roos & Boron, 1981).

T_4 was determined by using a modified Corning T_4 radioimmunoassay kit. The modification was that the standard curve was prepared with the perfusion medium and T_4 tracer was diluted to 20000 c.p.m./tube. The T_3 radioimmunoassay described by Hesch *et al.* (1974) was also modified by diluting standards in the perfusion medium and separating bound and free hormone by a second antibody (RD 17 anti-rabbit precipitating donkey serum; Wellcome, Beckenham, Kent, U.K.) (final dilution 1:550) in the presence of normal rabbit serum (final dilution 1:110) without further washing of the bound precipitated hormone. rT_3 was determined by a solid-phase radioimmunoassay (Kaminski *et al.*, 1980) and 3,3'- T_2 by a radioimmunoassay as described by Höffken *et al.* (1978).

Addition of perfusion medium (10–50 μl) resulted in parallel standard curves in the radioimmunoassays we used; no corrections for recovery in the presence of perfusion medium were necessary.

Preparation of microsomes before or after perfusion

This was performed as described by Dallner (1974). Microsomal membranes were washed with Tris buffer (0.15 mol/litre adjusted to pH 8.0 with HCl), resuspended in sucrose (0.25 mol/litre) and stored frozen at -20°C until used. L -Thyroxine 5'-deiodinase assay was performed as described by Auf dem Brinke *et al.* (1979), with the modification of elevating the dithioerythritol concentration to 10 mM.

Extraction of iodothyronines from portions of liver after freeze-clamping at the beginning or end of perfusion

This was performed, with minor modifications, by the method of Flock & Bollman (1955), in duplicate. Pieces of liver were homogenized in sucrose (0.25 mol/litre) as described above in the presence of 6-n-propyl-2-thiouracil (100 $\mu\text{mol}/\text{litre}$) to inhibit enzymic deiodination of iodothyronines. A portion (0.6 g) of liver corresponding to 3 ml of homogenate was repeatedly extracted with acid butanol (1×6 ml, 2×3 ml), the butanolic phases were combined, 6 ml of chloroform was added and iodothyronines were extracted into the aqueous phase with 12 ml of NH_3 (2 mol/litre) and this extraction was repeated twice with 3 ml of NH_3 . The aqueous phases were combined and freeze-dried overnight. The bright white material was dissolved in 1.5 ml of ethanol/0.05 M-NaOH (2:1, v/v), the precipitate formed overnight in the yellowish extract was spun down (10000 g) and then the volume of the supernatant was adjusted again to 1.5 ml. Extraction was performed by shaking stoppered glass tubes for 5 min at each step; phase separation was accelerated by centrifugation for 5 min at 2000 rev/min (r_{av} , 21.1 cm) in MSE Mistral 6L centrifuge. Routinely recovery of iodothyronines were run in parallel. Iodothyronines were determined in the ethanolic extracts by the radioimmunoassays described above with the modification of addition of alkaline ethanol to the standards instead of perfusion medium. Recovery of iodothyronines was parallel to iodothyronine standard curves in all the radioimmunoassays and no corrections were necessary (Takaishi *et al.*, 1978).

Intracellular pH

The intracellular pH was determined (Table 1) from the distribution of 5,5'-dimethyloxazolidine-2,4-dione, a non-metabolizable weak acid (pK 6.1), between the intra- and extra-cellular space, as described previously (Waddell & Butler, 1959; Soboll *et al.*, 1980). At the end of separate perfusion

Table 1. *Effect of different perfusate pH values on the pH in the liver cell*

The intracellular pH at the end of 2 h of perfusion time was measured. Values are given as means $\pm$ s.e.m. (number of livers). Concentrations of the dissociated form (DMO^-) of 5,5'-dimethyl-oxazolidine-2,4-dione are in mM. For calculations, see the Materials and methods section.

pH _{perfusate}	7.07 $\pm$ 0.01 (4)	7.43 $\pm$ 0.01 (4)	7.56 $\pm$ 0.02 (6)
[DMO^-] _{perfusate}	0.27	0.29	0.29
[DMO^-] _{cellular}	0.04 $\pm$ 0.01	0.09 $\pm$ 0.02	0.10 $\pm$ 0.02
ΔpH	0.91 $\pm$ 0.16	0.52 $\pm$ 0.06	0.46 $\pm$ 0.05
pH _{cellular}	6.16 $\pm$ 0.15	6.89 $\pm$ 0.11	7.10 $\pm$ 0.03

experiments, [$2\text{-}^{14}\text{C}$]5,5'-dimethyloxazolidine-2,4-dione (300 $\mu\text{mol/litre}$; 55 mCi/mol; New England Nuclear) and [^3H]inulin (New England Nuclear; 225 mCi/mg) were infused at a rate of 5 μCi and 20 $\mu\text{Ci/min}$ respectively for 5–7 min in an open non-circulating perfusion system; a steady state was attained between 3 and 7 min, where the radioactivities in the effluent became constant. At the end of this equilibration period, total [^{14}C]5,5'-dimethyloxazolidine-2,4-dione (dissociated and undissociated form) and [^3H]inulin were determined in the perfusate and liver samples after freeze-clamping (Sies *et al.*, 1973). The contents of tracer in the liver samples were referred to the protein content on the basis of the assumption of 3.8 μl of water/mg of cytosolic protein (Soboll *et al.*, 1980). The values obtained for the transmembrane pH difference at neutral pH are in good agreement with data published by Soboll *et al.* (1980).

Statistical evaluations

Students *t*-test was applied to compare the four groups of pH-dependent perfusion. Logarithmic disappearance curves of iodothyronines were analysed by least-squares analysis and by assuming one (T_3 and rT_3) or two (T_4) components of disappearance. The fast component between 1 and 10 min of disappearance of T_4 was corrected for the slow contribution represented by k_{+1} .

Results

Intracellular pH

As Table 1 shows, alterations in the pH of the perfusate resulted in corresponding pronounced pH changes within the liver cell, representing mainly those of the cytosolic compartment (Soboll *et al.*, 1980).

T_4 net uptake

T_4 disappeared from the medium during perfusion of isolated rat liver with the biphasic kinetic behaviour shown in Fig. 1. Iodothyronines were

Fig. 1. Disappearance curves of T_4 , T_3 and rT_3 from perfusion medium at pH 7.3 during 2 h of perfusion of isolated rat liver

T_4 (O, $n = 11$) exhibits two components of disappearance (extrapolated continuous lines). The values of initial disappearance were corrected for the slow component, and from this graph (□) k_2 was evaluated. Graphs for T_3 (Δ, $n = 6$) and rT_3 (●, $n = 3$) show only one component of disappearance. The lower limit of detection (l.d.) of radioimmunoassays is shown at the bottom of the graph.

determined at different time points by taking portions from the recirculating medium starting 1 min after addition of T_4 at zero time. Two components of disappearance were evaluated from these data by plotting the concentration difference of T_4 between 1 min after addition of T_4 and points x in time as a function of time: a fast component, representing presumably equilibration time with the liver, with half-life (t_1) of about 30 min, and a slower component with a t_1 of about 9 h (Table 2).

Table 2. *Effect of pH on half-lives of T₄, T₃ and rT₃ in perfused rat liver*

Disappearance rates were calculated assuming one (T₃ and rT₃) or two components of disappearance (T₄) by least-squares regression analysis between points in time between 1 and 10 min and between 20 and 120 min (T₄) or 1 and 120 min (T₃, rT₃).

Substrate	pH	10 ⁴ × k ₊₁	t _{1/2} (1) (min)	10 ⁴ × k ₊₂	t _{1/2} (2) (min)	No. of livers (n)
T ₄	7.15	12.2	567	158	44	3
	7.20	15.2	457	347	20	4
	7.37	11.4	606	277	25	11
	7.54	11.4	606	204	34	6
T ₃	7.12	13.5	513	—	—	3
	7.27	12.2	566	—	—	6
	7.47	15.4	449	—	—	3
rT ₃	7.35	796	8.7	—	—	3

Table 3. *Effect of different perfusate pH values on rat liver T₄ uptake, content and apparent conversion rate, and on T₃ content, output and net production*

Livers were perfused for 2 h with 26 nmol of T₄ in 200 ml of perfusate (initial concentration). Apparent conversion rate is defined as the ratio of T₃ net production to T₄ net uptake. Values are given as means ± s.e.m., and statistically significant differences compared with the pH 7.20 group are indicated: **P* < 0.02; †*P* < 0.01; ‡*P* < 0.005; §*P* < 0.001.

pH of perfusion medium	T ₄ net uptake (pmol/g)	T ₄ content (pmol/g)	T ₃ content (pmol/g)	T ₃ output (pmol/g)	T ₃ net production (pmol/g)	Apparent conversion rate (%)	No. of livers (n)
7.15 ± 0.01	766 ± 90	418 ± 57	18.1 ± 4.2	40.0 ± 2.3†	58.2 ± 3.4§	7.7	3
7.20 ± 0.01	1080 ± 199	319 ± 109	19.0 ± 4.5	107.5 ± 11.6	126.5 ± 11.1	13.0	4
7.37 ± 0.04	968 ± 94	417 ± 48	24.1 ± 2.8	65.5 ± 8.4*	85.5 ± 6.5‡	9.6	11
7.54 ± 0.05	825 ± 48	337 ± 42	14.9 ± 2.0	44.6 ± 4.1§	59.4 ± 5.2§	7.3	6

The change in perfusate T₄ concentration during the perfusion was used to determine the net liver T₄ uptake. As shown in Table 3, net uptake (9.16 nmol) is not dependent on the pH of perfusion in the pH range 7.1–7.6. The net uptake amounts to 36% of the initial T₄. The net T₄ uptake per total liver was not dependent on liver weight, but a statistically significant correlation was found between T₄ net uptake during perfusion (0.41–1.66 nmol of T₄/g of liver) and initial T₄ concentration (71–160 nmol of T₄/litre) [*r* = 0.61, *n* = 24 (for this evaluation, data from all livers were combined irrespective of pH), *P* < 0.001]. After 2 h of circulation of T₄ in perfusion medium without liver, we found a T₄ disappearance smaller than 3% and no biphasic kinetics were detectable. This demonstrates that the fast and slow components of T₄ net uptake are not caused by binding of T₄ to the fluorocarbon medium applied in the perfusion system itself. A similar finding was reported for media containing erythrocytes or albumin (Flock & Owen, 1965; Jennings *et al.*, 1979).

T₄ content

The total T₄ content of each liver was calculated

from the T₄ concentration measured in a small liver lobe before recirculating perfusion was started and that remaining in the liver at the end of the experiment. T₄ content increased from 46 pmol/g of liver to 380 pmol/g after 2 h of perfusion with medium containing initially 130 nmol of T₄/litre. As Table 3 shows, liver content of T₄ was also pH-independent after 2 h perfusion. However, the total content of T₄ found in the liver after perfusion was only 41% of the T₄ that disappeared from the perfusion medium, this ratio also being pH-insensitive. The content of T₄ was statistically significantly dependent on T₄ net uptake by the liver [*r* = 0.44, *n* = 24 (for this evaluation, data from all livers were combined irrespective of pH), *P* < 0.05].

Net T₃ uptake

Net T₃ uptake, calculated from the disappearance of T₃ from the perfusion medium as described above for T₄, was evaluated with only one component of disappearance (Fig. 1), owing to the small concentration differences. The mean *t*_{1/2} (8 h) seemed to be similar to that of T₄ in this model, but one has to consider that data were extrapolated from only 2 h of perfusion (Table 2). Total net uptake (0.64 nmol)

Table 4. Effect of different perfusate pH values on rat liver T₃ uptake, and T₄ and T₃ content
Livers were perfused for 2 h with 3 nmol of T₃ or rT₃ in 200 ml of perfusate (initial concentration). Values are given as means ± S.E.M.

Substrate	pH of perfusion medium	T ₄ content (pmol/g)	T ₃ net uptake (pmol/g)	T ₃ content (pmol/g)	No. of livers (n)
T ₃	7.12 ± 0.01	11.4 ± 1.2	72.6 ± 17.6	49.8 ± 4.3	3
T ₃	7.27 ± 0.01	16.0 ± 1.6	60.6 ± 2.5	58.3 ± 5.1	7
T ₃	7.47 ± 0.02	17.6 ± 2.1	62.3 ± 7.1	55.5 ± 4.6	3
rT ₃	7.35 ± 0.05	15.4 ± 1.5	—	2.5 ± 0.1	7

representing 21% of initially added T₃ (15 nmol/litre) was less than that of T₄ and also independent of pH (Table 3).

T₃ content

The T₃ content of the liver was determined as for T₄. It increased from 6 pmol/g of liver to 54 pmol/g after 2 h of perfusion with an initial concentration of T₃ of 15 nmol/litre of perfusion medium. Of the T₃ initially offered, 21% disappeared from the perfusate, and 85% of this amount was recovered as liver content after 2 h of perfusion, indicating that degradation of T₃ initially offered was less than 3% (~100 pmol) during 2 h of perfusion. The T₃ content was not dependent on pH of perfusion, as is shown in Tables 3 and 4.

Degradation of endogenous liver T₃ was also determined where liver was perfused only with rT₃. The T₃ content of liver decreased from an initial 6.0 pmol/g to 2.5 pmol/g, but no T₃ or 3,3'-T₂ output into the medium was detectable under these conditions. This means that only 35 pmol of T₃ was degraded at the low endogenous T₃ substrate concentration.

rT₃ net uptake

rT₃ offered in initial concentrations equal to those of T₃, i.e. 15 nmol/litre of perfusion medium, disappeared extremely rapidly from the perfusate; after 60 min, rT₃ was no longer detectable in the perfusion medium (Fig. 1). The estimated *t*₁ of 7 min is much shorter than that of T₄ and T₃ disappearance and shorter than the calculated equilibration time of T₄ (Table 2). Similar fast kinetics were reported for rT₃ by Flock & Owen (1965) and van der Heide (1980). The question of rT₃ degradation and its dependence on the pH of perfusion cannot be answered by our recirculating system. No significant amounts of rT₃ were detectable in the liver before or after 2 h of perfusion with either T₄, or T₃ and rT₃ (detection limit 75 fmol/g of liver). rT₃ added to homogenate before extraction was recovered with 90–100% (*n* = 5) in the extract.

Fig. 2. Effect of different perfusate pH values on hepatic output of T₃

The initial T₄ concentration was 26 nmol/200 ml of perfusate. Data are given as means or means ± S.E.M. (120 min). □, pH 7.15, *n* = 3; ●, pH 7.20, *n* = 4; ○, pH 7.37, *n* = 11; △, pH 7.54, *n* = 6. Statistical analysis: T₃ output was in all cases statistically significantly different (*P* < 0.05) at 30 min from zero-time values. At 120 min of perfusion time, T₃ output at pH 7.20 was elevated compared with pH 7.15 (*P* < 0.01), pH 7.35 (*P* < 0.02) and pH 7.54 (*P* < 0.001).

T₄ 5'-deiodination in the perfused rat liver

Deiodination of T₄ to T₃ by L-thyroxine 5'-deiodinase could be demonstrated by the output of

Fig. 3. Effect of different perfusion-medium pH values on hepatic net production of T_3 .

The initial T_4 concentration in the perfusate was 26 nmol/200 ml; the perfusion time was 2 h. Least-squares analysis of individual T_3 production (O) resulted in a statistically significant correlation ($r = -0.79$, $n = 21$, $P < 0.001$). Livers perfused at pH 7.15 (Δ) were not considered for statistical evaluation since T_3 production decreases below pH 7.17.

T_3 into the perfusate and increased T_3 content of liver when T_4 was offered as substrate for deiodination experiments. The kinetic behaviour of T_3 output is presented in Fig. 2. Significant output of T_3 was detectable after 30 min ($P < 0.05$) and remained linear for up to 120 min. This pattern was comparable with data reported by Flock & Owen (1965), Hesch *et al.* (1975) and Jennings *et al.* (1979). Interestingly, however, T_3 output by the perfused rat liver was dependent on the pH of perfusion (Figs. 2 and 3). T_3 output was significantly higher at pH 7.20 compared with pH 7.15 ($P < 0.01$), pH 7.37 ($P < 0.02$) and pH 7.54 ($P < 0.001$).

The T_3 content in the perfused liver was slightly but not significantly lower at pH 7.54 than at other pH values (cf. Table 3), whereas both parameters were not significantly different between pH 7.15 and 7.37. The T_3 content of total liver after 2 h of perfusion was one third compared with T_3 in the medium due to T_3 output by the liver during perfusion. Table 3 demonstrates that T_3 net production, i.e. the sum of T_3 output and liver content, was dependent on pH of perfusion too. T_3 net productions at pH 7.15 and 7.54 were not significantly different from each other, but T_3 net production at pH 7.20 was significantly higher than at pH 7.37 ($P < 0.005$), and at both pH values net T_3 production

was statistically significantly elevated compared with pH 7.15 and 7.54. The same conclusion could be drawn from the apparent conversion rate of 7–13% defined by the ratio of T_3 net production divided by T_4 uptake and thus for the activity of L-thyroxine 5'-deiodinase in the isolated perfused rat liver, since T_4 net uptake was not dependent on pH of perfusion and T_3 content was only slightly lower in the 'alkaline' group; this indicates that efflux of T_3 seemed not to be a pH-regulated process. The correlation between individual values of T_3 production and pH of perfusion is shown in Fig. 3. Values higher than pH 7.17 gave a statistically significant ($r = -0.79$, $n = 21$, $P < 0.001$) correlation. Below pH 7.17, T_3 production decreased again.

Microsomal fractions prepared after 2 h of perfusion still showed the same L-thyroxine 5'-deiodinase activity (24 pmol of T_3 formed/min per mg of protein) as comparable fractions prepared without prior perfusion; i.e., perfusion did not, in itself, influence the microsomal deiodinase activity.

L-3,3',5'-Tri-iodothyronine 5'-deiodination by perfused rat liver

No significant net production of rT_3 or 3,3'- T_2 from T_4 in the perfusate by intact liver was detectable. However, very active deiodination of rT_3 to 3,3'- T_2 was demonstrated in perfused rat liver (Fig. 4). Owing to the rapid degradation, only concentrations of 3,3'- T_2 in the medium are given in Fig. 4. The very low levels of 3,3'- T_2 in the perfusate found after 10–30 min were therefore probably due to the rapid degradation of this intermediary

Fig. 4. Appearance of 3,3'- T_2 in the perfusion medium after addition of rT_3 .

The initial concentration of rT_3 was 3 nmol/200 ml of perfusate. Values are given as means $\pm$ S.E.M. ($n = 3$). The lower limit of detection (shaded) is shown at the bottom of the graph.

metabolite of iodothyronines, a result also demonstrated by Flock & Owen (1965) in tracer studies. No significant rT_3 and 3,3'- T_2 contents in liver before and after 2 h of perfusion were detectable in a recirculating perfusion system; this may be also due to the fast degradation of rT_3 and 3,3'- T_2 .

Discussion

The experiments demonstrate that iodothyronines are taken up, metabolized and mono-deiodinated by the isolated perfused rat liver. Moreover, the characteristics of these reactions are similar to those previously shown *in vivo* and *in vitro* [for a review, see Hesch (1981)]. Furthermore T_3 production by the iodothyronine 5'-deiodinase depends on the intracellular pH and decreased with increasing pH in the perfusion medium within the physiological range (Fig. 3). Determination of the intracellular pH (Table 1), representing mainly the pH of the cytosolic compartment (Soboll *et al.*, 1980), allows the conclusion that the pH optimum *in vitro* of 6.5–6.9 of the L-thyroxine 5'-deiodinase of the endoplasmatic reticulum (Auf dem Brinke *et al.*, 1979; Fekkes *et al.*, 1979), which is a cytosolic orientated membrane-integrated enzyme (Köhrle & Hesch, 1979), corresponds well with the optimum of T_3 production of liver perfused with medium of pH 7.2, which results in an intracellular pH of about 6.5. In contrast, hepatic content and net uptake of T_4 and T_3 are not dependent on pH (compare Tables 3 and 4) and agree well with previously published data from experiments using different perfusion systems (Flock & Owen, 1965; Hillier, 1972; Hesch *et al.*, 1975; Jennings *et al.*, 1979; Pardridge & Mietus, 1980; Carter *et al.*, 1979). One major discrepancy between our model and some of those reports is the low disappearance and/or degradation of T_3 .

This could be the reason for the higher output and production rates of T_3 (7–13%) than those reported previously (Hesch *et al.*, 1975; Jennings *et al.*, 1979). Characteristics of Fluorocarbon 43 medium could give one explanation, but up to now no data on binding properties of the Fluorocarbon 43 medium for iodothyronines are available.

The finding of pH-dependent T_3 production in the intact organ, where uptake by the liver as well as transport and binding of iodothyronines by proteins of membranes and cytosol could interfere with the enzymic process, is remarkable, since it could indicate a further regulatory mechanism apart from diminished uptake of the substrate T_4 shown for 'starved' livers by Jennings *et al.* (1979) or decreased enzyme activity itself in livers of streptozotocin-induced diabetic rats (Jennings, 1981). However, we do not know from our experiments if cellular changes in pH directly or indirectly regulate

activity of iodothyronine 5'-deiodinase. A possible mechanism could be the pH-dependent production of regulatory iodothyronines rT_3 and/or 3'5'- T_2 , which were shown to inhibit T_3 production by the L-thyroxine 5'-deiodinase in pH-dependent manner in microsomal fractions *in vitro* (Kaminski *et al.*, 1981).

Hepatic T_4 uptake from our medium, which is free of binding protein, was similar to that in livers perfused with solutions containing binding proteins for iodothyronines such as, for example, albumin. The hypothesis of Hillier (1972) and Pardridge & Mietus (1980), that free iodothyronines are taken up by the liver and uptake is not influenced by iodothyronine-binding proteins of serum is thus supported. We found a correlation between the initial T_4 concentration of the perfusion medium and net uptake and also to hepatic T_4 content. This is in good agreement with the findings of Jennings *et al.* (1979) for this range of T_4 concentrations which is similar to that found in rat serum. Correction for iodothyronine content in the extracellular space of rat liver determined by [3H]inulin after the end of perfusion did not qualitatively change the distribution pattern of iodothyronines between medium and tissue. The contents of both T_4 and T_3 as given in Table 3 were reduced by only 8–12%. This demonstrates that liver cells concentrated T_4 3–4- and T_3 4–7-fold from the medium at the end of perfusion, irrespective of pH. Extrapolation of the apparent T_3 production rate of perfused liver to daily production of T_3 by the liver *in situ* yields a value of about 11 nmol. Taking into account the aforementioned T_3 degradation of 3%, this value is negligibly higher. The activity of the microsomal enzyme (without addition of exogenous dithioerythritol *in vitro*) could contribute 23 nmol of T_3 per day. Estimation of T_3 production by multiplying endogenous liver content of T_4 (0.45 nmol) by the apparent conversion rate of T_4 (10% in 2 h) resulted in a production of 0.65 nmol of T_3 per day. Moreale de Escobar & Moreale del Rey (1962) determined a daily T_3 production of 0.9 nmol by isotopic-equilibrium methods, which is within the range of our estimation from the deiodination rate. However, our experiments also demonstrated that the liver can produce a more-than-10-fold amount of daily production of T_3 from T_4 , and L-thyroxine 5'-deiodinase is not yet saturated. Extrapolation of T_4 degradation from the slow component of disappearance yields a turnover rate of 23–31 nmol/day, which is also within the range of the production rate *in vitro* and higher than that of T_3 *in vivo*. The same conclusion can be drawn from the apparent conversion rate of T_3 from T_4 . This finding is somewhat in contrast with a preliminary report of Distefano (1981), which seems to indicate that most of the T_3 originates from extrahepatic T_4 deiodination.

The fact that we cannot also detect rT_3 net production by the perfused rat liver in alkaline-pH medium, where L-thyroxine 5-deiodinase activity increases *in vitro*, could result from a very high activity of iodothyronine 5'-deiodinase compared with iodothyronine 5-deiodinase in this perfusion model, as was suggested from the transient $3,3'$ - T_2 production (Fig. 3) that was found also in the earlier report of Flock & Owen (1965). Our finding of a very low degradation rate of T_3 , which is only a poor substrate for iodothyronine 5'-deiodinase (J. Köhrle, unpublished work) but a substrate as good as T_4 for iodothyronine 5-deiodinase microsomal fractions *in vitro* (Visser *et al.*, 1979), supports this hypothesis. However, we cannot exclude that rT_3 , intermediately formed from T_4 by 5-deiodinase, is rapidly further metabolized by $3(5)$ -deiodination to $3',5'$ - T_2 etc., thus accounting for a part of T_4 degradation. Since up to now no report could convincingly demonstrate significant circulating rT_3 serum levels in adult rats, we suggest from our perfusion data that rT_3 , if formed at all by the rat liver from T_4 via 5-deiodination, is so rapidly metabolized by 5'-deiodinase within the hepatocyte that no output occurs in normal situation. If rT_3 levels should prove to be measurable in rat serum their origin might be in extrahepatic tissues. Intact rat liver seems to represent an organ with predominating iodothyronine 5'-deiodinase activity. The observation of rT_3 production by iodothyronine 5-deiodinase in the homogenate or microsomal fraction may be due to artificial conditions *in vitro*, where a disturbed cellular regulation may be found owing to, for example, lost compartmentalization. Accordingly, administration *in vivo* of pharmaceutical doses of rT_3 to rats only slightly decreased circulating T_3 levels (Coiro *et al.*, 1980). Therefore the physiological importance of the finding *in vitro* of the pH-dependent competitive inhibition of L-thyroxine 5'-deiodinase by the regulatory active iodothyronines rT_3 and $3',5'$ - T_2 has to be demonstrated.

Taken together, our results provide strong evidence that L-thyroxine 5'-deiodination to T_3 is, beside other factors {uptake and content of substrate (Jennings *et al.*, 1979), cellular redox and glutathione status (Visser, 1981), activity of the enzyme itself (Jennings, 1981), ratio of 5'- to 5-deiodinase activity (Köhrle *et al.*, 1979; Cheron *et al.*, 1980) [for reviews, see Hesch (1981) and Werner (1981)]}, regulated by the pH of perfusion of isolated rat liver.

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