Thyroxine action on the rat liver nuclear thyroid-hormone receptors

Binding of thyroxine to the nuclear non-histone protein and induction of mitochondrial α -glycerophosphate dehydrogenase activity

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The possibility that thyroxine (T_4) itself exerts the hormonal effect *in vivo* on the rat liver nuclear receptor was studied with the aid of iopanoic acid (IOP), an inhibitor of the conversion of T_4 into tri-iodothyronine (T_3). After administration of $2.4\text{ }\mu\text{g}$ of $T_4/100\text{ g}$ body weight to hypothyroid rats for 7 days, T_4 and T_3 concentrations in serum and in the liver nuclear non-histone protein (NHP) were all increased to the hyperthyroid range. Hepatic mitochondrial α -glycerophosphate dehydrogenase (α -GPD) activity and DNA content increased significantly. The equilibrium association constant (K_a) of the nuclear T_3 receptor was unchanged and the maximal binding capacity (C_{max}) increased 1.4-fold. Simultaneous administration of IOP ($5\text{ mg}/100\text{ g}$ body weight) to the rats given $2.4\text{ }\mu\text{g}$ of $T_4/100\text{ g}$ body weight completely blocked the conversion into T_3 . The serum T_4 was even more increased, whereas the serum T_3 decreased to the hypothyroid range. Although the NHP-bound T_4 was at a concentration comparable with the rats given T_4 alone, no NHP-bound T_3 was detected. Yet the α -GPD activity was elevated 2.8-fold and the DNA content increased to the same extent as observed in the rats given T_4 alone. The K_a and C_{max} of the nuclear receptor were significantly decreased. After administration of 48 or $480\text{ }\mu\text{g}$ of $T_4/100\text{ g}$ body weight for 3 days, serum T_4 and T_3 were markedly increased. The NHP-bound T_3 was also increased, but no NHP-bound T_4 was detected. The α -GPD activity was markedly elevated, but the DNA content was unchanged. The C_{max} per g of liver was increased, whereas the K_a remained unchanged. Simultaneous administration of IOP to these animals could not completely block the T_4 conversion. The observed hormonal effects in the absence of nuclear T_3 indicate that T_4 possesses the intrinsic hormonal activities on the rat liver. T_4 is less potent in induction of α -GPD activity but as potent in increment of hepatic DNA as T_3 . Although the binding site for T_4 is not fully characterized, it appears to be acidic NHP. T_4 is an active hormone, yet is also a prohormone of T_3 , offering the closest analogy with testosterone.

T_4 accelerates growth and development and increases the activities of a variety of enzymes (Tata, 1974). It is generally believed that these hormonal effects are initiated by the binding of T_3 , largely produced from monodeiodination of T_4 in peripheral tissues, to nuclear receptors (Ingbar & Braverman, 1975; Oppenheimer *et al.*, 1976; Samuels, 1978; Chopra *et al.*, 1978). Accordingly, it has been suggested that T_4 is a prohormone which needs to be converted into T_3 before exerting the hormonal

effects (Ingbar & Braverman, 1975; Oppenheimer *et al.*, 1972a).

However, the biochemical role of T_4 in thyroid-hormone action remains unsettled. The binding affinity of T_4 for the nuclear receptor is one-tenth that of T_3 (Oppenheimer *et al.*, 1976). Only 10–15% of the iodothyronine bound to the receptor in intact rats is T_4 (Surks & Oppenheimer, 1977), possibly reflecting non-specific binding (Oppenheimer *et al.*, 1976). T_4 did not suppress serum thyrotropin when the conversion into T_3 was blocked by IOP (Larsen *et al.*, 1979; Obregon *et al.*, 1980). These results suggest that T_4 may be an inactive prohormone. Conversely, euthyroidism is largely maintained by T_4 in the low- T_3 states found in various non-

Abbreviations used: T_4 , thyroxine; T_3 , tri-iodothyronine; IOP, iopanoic acid; i.p., intraperitoneally; s.c., subcutaneously; α -GPD, α -glycerophosphate dehydrogenase; NHP, non-histone protein.

thyroidal illnesses (Chopra *et al.*, 1975). Similarly T_4 -enriched incubation media increase 3-fold the growth rate of GH₁ cells in the absence of $T_4 \rightarrow T_3$ conversion (Samuels *et al.*, 1979). The mechanism of T_4 action, however, has not been defined.

In order to elucidate this mechanism in peripheral tissues, 6-n-propylthiouracil-inhibited T_4 conversion was studied previously (Oppenheimer *et al.*, 1972a; Frumess & Larsen, 1975). However, because T_4 conversion was inhibited by only 60%, the results were inconclusive. In the present study we employed iopanoic acid, a more potent inhibitor of T_4 conversion, to examine the intrinsic hormonal activity of T_4 .

The maximal binding capacity ($C_{\max}$) (Samuels *et al.*, 1976, 1977; Burman *et al.*, 1977; DeGroot *et al.*, 1977; Schussler & Orlando, 1978; Dillmann *et al.*, 1978; Dillmann & Oppenheimer, 1979; Hamada *et al.*, 1979; Nakamura *et al.*, 1979a,b) and the equilibrium association constant (K_a) (Bernal *et al.*, 1978) of nuclear receptors are altered under various pathophysiological conditions, although their involvement in hormone action is not fully understood. We have previously demonstrated that $C_{\max}$ was increased 2.5-fold after the administration of excess T_3 to hypothyroid rats (Hamada *et al.*, 1979; Nakamura *et al.*, 1979a,b). The K_a was not affected when corrected for endogenous T_3 bound to the receptor. In the present study we examine the effects of T_4 on the $C_{\max}$ and K_a of the rat liver nuclear receptor.

Materials and methods

Experimental animals

Wistar-strain male rats weighing about 150 g were obtained from Shimizu Laboratory Animal Co. (Kyoto, Japan), and raised at 25°C on a low-iodine rat diet (Oriental Animal Foods Co., Osaka, Japan). After several days the rats were thyroidectomized and supplemented with 0.5% $CaCl_2$ in the drinking water. At about 1 week after thyroidectomy the rats were injected i.p. with ^{131}I (100 μCi /100 g body weight). The animals were weighed daily and were used only if their weight gain was less than 5 g per week.

Two series of experiments were performed. In the first, two groups of the hypothyroid rats were injected s.c. with 2.4 μg of T_4 /100 g body weight, together with IOP 5 mg/100 g body weight or with vehicle for 7 days. In the second series, five groups of the animals were injected s.c. with various doses of T_4 with or without IOP: Group 1, injected s.c. with the vehicle alone (thyroidectomized + vehicle); Group 2, injected s.c. with 48 μg of T_4 /100 body weight for 3 days (thyroidectomized + 48 μg of T_4); Group 3, injected with both 48 μg of T_4 and IOP for 3 days (thyroidectomized + 48 μg of T_4 + IOP);

Group 4, injected with 480 μg of T_4 /100 g body weight for 3 days (thyroidectomized + 480 μg of T_4); Group 5, injected with both 480 μg of T_4 and IOP for 3 days (thyroidectomized + 480 μg of T_4 + IOP). Each animal was given half the dose of T_4 in the morning and half in the evening. IOP was dissolved in 0.05 M-NaOH followed by neutralization with HCl to pH 7.8, and 5 mg/100 g body weight injected i.p. 12 h before each T_4 injection.

Preparation of nuclear extracts

The animals were killed by cardiac puncture under light ether anaesthesia and the livers perfused *in situ* with 20 ml of cold 0.9% NaCl. The excised liver was minced with scissors, and washed repeatedly with excess cold 0.9% NaCl and finally with 0.25 M-sucrose/3 mM-MgCl₂ solution. About 1 g of the liver was saved for determination of mitochondrial α -GPD activity. The minced liver was homogenized in 4 vol. of 0.25 M-sucrose/3 mM-MgCl₂ with a Teflon-pestle homogenizer. The homogenate was centrifuged at 700 g for 10 min. The pellet was resuspended in 2.4 M-sucrose/3 mM-MgCl₂ and centrifuged at 80 000 g ($r_{\max}$) for 45 min. To avoid possible contamination with T_4 from either the cytosol or the nuclear outer membrane, the nuclei were washed carefully in 20 ml of 0.25 M-sucrose/3 mM-MgCl₂ initially with, and subsequently without, 0.5% Triton X-100. A 1 ml portion of the last nuclear suspension was saved for determination of DNA. The final nuclear pellet was suspended in the extraction buffer (0.4 M-KCl/5 mM-MgCl₂/0.05 M-Tris/glycine buffer, pH 8.5) at a ratio of 1 g equivalent of liver nuclei per ml and gently stirred at 4°C for 1 h. Vigorous mixing resulted in decrease in T_3 binding by the nuclear extract. After centrifugation at 106 800 g ($r_{\max}$) for 30 min, the KCl extract was supplemented with 1 mM-dithiothreitol and stored at -20°C. The preparations were stable for 3 weeks under these conditions.

Determination of K_a and $C_{\max}$

The K_a and $C_{\max}$ were determined as described by Hamada *et al.* (1979) and Nakamura *et al.* (1979a). Briefly, the nuclear extract (0.1 ml) was supplemented with 50 μl of 25 mM-dithiothreitol and increasing amounts of [^{125}I] T_3 (0.01–0.1 pmol/tube; sp. radioactivity, 500–700 $\mu Ci/\mu g$; Dainabot Laboratories, Tokyo, Japan) with or without unlabelled T_3 (0.1–0.2 pmol/tube), and incubated at 20°C for 3 h. After the incubation, unbound hormone was separated by adding 1 ml of 2% (w/v) Dowex I (Cl⁻ form; 1X8, 200–400 mesh; Dow Chemical Co., Midland, MI, U.S.A.) anion-exchange resin in the extraction buffer followed by centrifugation at 1200 g for 20 min. The supernatant and resin pellet were counted for ^{125}I radioactivity. Scatchard plots were performed with and without

correction for endogenous T_3 binding to the nuclear NHP. The total amount of T_3 was the sum of the added T_3 and the endogenous T_3 measured by radioimmunoassay described below. Although T_4 was also bound to the nuclear NHP, it was not specifically corrected for. Because the affinity of T_4 for the nuclear receptors is one-tenth that of T_3 , it was not expected to interfere with the binding of added T_3 to the receptors.

Radioimmunoassay for T_3 in serum and nuclear extracts

Serum T_3 concentration was determined by the method described previously (Nakamura *et al.*, 1979a). A 0.1 ml portion of serum was used for the radioimmunoassay. The lowest detectable concentration was 20 pg/0.1 ml. Hormone-free rat serum, prepared by using anion-exchange-resin adsorption, was used to construct the standard curve.

The T_3 concentration in nuclear NHP was determined by the same method as that described previously (Nakamura *et al.*, 1979a). T_3 was extracted from the nuclear NHP (0.1–0.5 ml) by using a small Sephadex column equilibrated with 0.1 M-NaOH and was determined by a specific radioimmunoassay. The recovery of T_3 was estimated by adding a tracer dose of T_3 in advance to each sample. The lowest detectable T_3 concentration was 2 pg/0.1 ml of nuclear extract. Values given for the endogenous T_3 concentration were the means of duplicate determinations.

Radioimmunoassay for T_4 in serum and nuclear extracts

Serum T_4 concentration was determined by a radioimmunoassay kit for T_4 (SPAC- T_4 RIA kit, Mallinckrodt Co., St. Louis, MO, U.S.A.). A 25 μ l portion of serum or standard and 1.0 ml of the labelled T_4 solution were added successively to the T_4 -antibody-coated test tube and incubated for 1 h at 37°C. After aspiration of the contents, radioactivity bound to the test tube was counted.

T_4 concentration in the nuclear extract was determined by the method described below. A mixture of the nuclear extract or standard T_4 (0.2 ml), [125 I] T_4 (0.1 ml), and 95% ethanol (0.6 ml) was prepared in duplicate, and the radioactivity (T_0) counted to facilitate correction for recovery. The mixtures were incubated at 4°C overnight with shaking, followed by centrifugation at 1200g for 20 min. A 0.6 ml portion of the supernatant was counted for radioactivity (T), and then added to the extraction buffer (1.2 ml), 0.1% bovine γ -globulin/0.01 M-NaOH (0.2 ml), 0.01 M-NaOH (0.3 ml) and the diluted antiserum (0.1 ml). After incubation at 26°C overnight and at 4°C for 4 h, 0.5 ml of 4% (w/v) Dowex I anion-exchange resin in the extraction buffer was added to separate unbound hormone.

After centrifugation at 1200g for 20 min, the supernatant and resin pellet were counted for radioactivity. T_4 values thus obtained were corrected for the recovery of [125 I] T_4 (T/T_0) in each sample. The recovery ranged from 60 to 65%, and the lowest detectable concentration of T_4 was 10 pg/0.1 ml of nuclear extract.

Other assays

Mitochondrial α -GPD activity was measured by the method of Lee & Lardy (1965). Protein concentration was determined by biuret reaction with bovine serum albumin as standard. DNA was extracted by the method of Schmidt-Schneider (Schneider, 1946), and measured by Burton's (1956) method.

Results

To eliminate the potential effects of endogenous hormone, rats were rendered hypothyroid by total thyroidectomy and subsequent administration of 100 μ Ci of 131 I. Weight gain was less than 5 g per week and was not significantly changed after daily injection of vehicle alone. Serum T_4 and T_3 concentrations were $0.8 \pm 0.2 \mu\text{g}/100 \text{ ml}$ (mean $\pm$ s.d.) and $15.1 \pm 5.3 \text{ ng}/100 \text{ ml}$ respectively. Neither T_4 nor T_3 were detectable in the liver nuclear extract (Table 1) and liver mitochondrial α -GPD activity was markedly decreased. Liver nuclear T_3 receptor exhibited a K_a of $12.6 \pm 0.9 \times 10^9 \text{ M}^{-1}$ and a C_{max} of $679.7 \pm 82.7 \text{ pg of } T_3/\text{g of liver or } 219.5 \pm 30.5 \text{ pg of } T_3/\text{mg of DNA}$.

In the first series of experiments these animals were injected s.c. daily with 2.4 μg of $T_4/100 \text{ g}$ body weight with either IOP (5 mg/100 g body weight) or its vehicle for 7 days. After administration of 2.4 μg of T_4 in vehicle, serum T_4 and T_3 concentrations were increased respectively to $13.2 \pm 3.0 \mu\text{g}/100 \text{ ml}$ and $170.5 \pm 35.5 \text{ ng}/100 \text{ ml}$, and the T_4 and T_3 concentrations in the liver nuclear extract, presumably NHP-bound T_4 and T_3 , were increased to $0.286 \pm 0.101 \text{ ng}/\text{mg of DNA}$ and $0.355 \pm 0.140 \text{ ng}/\text{mg of DNA}$. The mitochondrial α -GPD activity was augmented 4.4-fold. The DNA content was significantly ($P < 0.02$) increased. Changes in the K_a and C_{max} of the liver nuclear T_3 receptor were also noted (Table 2). The uncorrected value of the K_a was markedly decreased, but the uncorrected value of the C_{max} was not altered significantly. Both determinations are thought to underestimate the actual values because the concentration of unlabelled NHP-bound T_3 generated from the injected T_4 is high enough to distort the Scatchard plot if uncorrected (Hamada *et al.*, 1979). After correction for the presence of unlabelled endogenous T_3 , the K_a of the T_4 -injected animals remained unchanged, whereas the C_{max} increased 1.4-fold when expressed

Table 1. T_4 and T_3 concentrations in serum and in the liver nuclear NHP, the liver mitochondrial α -GPD activity and DNA content in hypothyroid rats given various doses of T_4 with and without IOP

The values are the means for the number of animals used. The means of the two separate groups are statistically, a-a, insignificant ($P > 0.05$); and significant at the levels indicated; b-b (see Table 2), 0.05; c-c, 0.02; d-d, 0.001. The values are statistically: §, insignificant ($P > 0.05$) and significant at the levels indicated: *, 0.05; **, 0.02; †, 0.01 (see Table 2); ††, 0.001, compared with the hypothyroid animals [thyroidectomized (Tx) + vehicle]. Abbreviation used: n.d., not detectable.

Group	No. of animals	[Serum T_4] ($\mu\text{g}/100\text{ml}$)	[Serum T_3] (ng/100ml)	$10^{-3} \times \alpha\text{-GPD}$ activity ($\Delta A/\text{min per mg of protein}$)	DNA (mg in nuclei isolated from 1 g of liver)	T_4 in NHP (ng/mg of DNA)	T_3 in NHP (ng/mg of DNA)
Tx + 2.4 μg of T_4 , 7 days	6	$13.2 \pm 3.0^{a††}$	$170.5 \pm 35.5^{d††}$	$78.9 \pm 23.0^{c††}$	$4.10 \pm 0.50^{a***}$	0.286 ± 0.101^a	0.355 ± 0.140^d
Tx + 2.4 μg of T_4 + IOP, 7 days	6	$15.9 \pm 4.3^{a††}$	$37.8 \pm 21.7^{d§}$	$49.2 \pm 18.6^{c**}$	$4.29 \pm 0.69^{a**}$	0.240 ± 0.135^a	n.d. ^d
Tx + 48 μg of T_4 , 3 days	6	21.3 ± 4.3	303.0 ± 61.4	$105.6 \pm 26.6^{††}$	$3.55 \pm 0.76^{§}$	n.d.	0.352 ± 0.071
Tx + 48 μg of T_4 + IOP, 3 days	2	30.9	68.5	134.3	3.28	0.336	0.206
Tx + 480 μg of T_4 , 3 days	6	33.3 ± 10.8	659.2 ± 214.4	$175.5 \pm 32.9^{††}$	$3.31 \pm 0.21^{§}$	n.d.	0.575 ± 0.168
Tx + 480 μg of T_4 + IOP, 3 days	2	36.0	189.0	179.6	4.00	0.245	0.219
Tx + vehicle, 3 days	4	0.8 ± 0.2	15.1 ± 5.3	17.8 ± 4.5	3.14 ± 0.51	n.d.	n.d.

Table 2. Equilibrium association constant and maximal T_3 -binding capacity of the liver nuclear receptors with and without correction for endogenous T_3 in hypothyroid rats given various doses of T_4 with and without IOP

For an explanation of superscripts, footnote symbols and abbreviations, see Table 1.

Group	No. of animals	Uncorrected			Corrected		
		$10^9 \times K_a$ (M^{-1})	C_{max} (pg of T_3/g of liver)	C_{max} (pg of T_3/mg of DNA)	$10^9 \times K_a$ (M^{-1})	C_{max} (pg of T_3/g of liver)	C_{max} (pg of T_3/mg of DNA)
Tx + 2.4 μg of T_4 , 7 days	6	$5.4 \pm 1.9^{a††}$	$708.8 \pm 177.1^{c§}$	$178.8 \pm 65.9^{b§}$	$10.8 \pm 2.6^{b§}$	$951.3 \pm 126.7^{d†}$	$236.9 \pm 57.1^{d§}$
Tx + 2.4 μg of T_4 + IOP, 7 days	6	$7.5 \pm 2.1^{a†}$	$458.0 \pm 91.8^{c†}$	$107.3 \pm 13.7^{b††}$	$7.5 \pm 2.1^{b†}$	$458.0 \pm 91.8^{d†}$	$107.3 \pm 13.7^{d††}$
Tx + 48 μg of T_4 , 3 days	6	$6.4 \pm 1.7^{††}$	$902.8 \pm 177.4^*$	$264.1 \pm 51.4^{§}$	$12.6 \pm 0.4^{§}$	$1082.7 \pm 230.5^{**}$	$312.8 \pm 36.9^{†}$
Tx + 48 μg of T_4 + IOP, 3 days	2	7.6	709.8	216.8	12.4	839.0	258.0
Tx + 480 μg of T_4 , 3 days	6	$6.5 \pm 2.2^{††}$	$907.3 \pm 147.0^*$	$275.0 \pm 51.1^{§}$	$12.6 \pm 2.9^{§}$	$1456.6 \pm 243.9^{††}$	$445.0 \pm 99.9^{†}$
Tx + 480 μg of T_4 + IOP, 3 days	2	5.8	1244.8	312.4	11.6	1307.3	328.1
Tx + vehicle, 3 days	4	12.6 ± 0.9	679.7 ± 82.7	219.5 ± 30.5	12.6 ± 0.9	679.7 ± 82.7	219.5 ± 30.5

per g of liver and to a comparable degree when expressed per mg of DNA.

In order to inhibit peripheral conversion of the injected T_4 into T_3 , IOP (5mg/100g body weight) was simultaneously administered to the rats given 2.4 μg of T_4 for 7 days. In these animals the serum T_4 concentration increased, but the serum T_3 concentration decreased to hypothyroid levels

(Table 1). No T_3 was detected in the liver nuclear NHP, although NHP-bound T_4 was present at the same concentration. Notably the α -GPD activity remained in the high-normal range, and the DNA content was significantly ($P < 0.05$) higher than in the hypothyroid animals.

In the IOP-treated rats, the nuclear T_3 receptor showed marked changes in K_a and C_{max} , which

differed from those noted in rats given T_4 alone. Both the K_a and the C_{max} decreased significantly ($P < 0.05$). The C_{max} decreased to approx. one-half that of the hypothyroid animals (Table 2).

In the second series of experiments, hypothyroid rats were injected s.c. with 48 or 480 μg of T_4 /100 g body weight for 3 days. Serum T_4 and T_3 concentrations, T_3 concentrations in the nuclear extract and α -GPD activities all increased to markedly hyperthyroid levels (Table 1). Notably, no T_4 was detectable in the nuclear extract, despite the very large doses of injected T_4 . The DNA contents expressed per g of liver were not changed significantly (Table 1).

The K_a values were unchanged when corrected for endogenous T_3 , though markedly decreased when uncorrected (Table 2). The corrected values of the C_{max} per g of liver were increased 1.6-fold and 2.1-fold in these two groups of animals compared with the hypothyroid rats. These results were commensurate with previous observations made on rats after injection of large doses of T_3 (Hamada *et al.*, 1979; Nakamura *et al.*, 1979a,b).

IOP (5 mg/100 g body weight) was injected into rats given 48 or 480 μg of T_4 /100 g body weight for 3 days. It was observed that IOP did not completely block the conversion of T_4 into T_3 in the rats given these high doses of T_4 . Serum T_4 concentrations even increased, whereas the serum T_3 concentrations markedly decreased (Table 1). Both T_4 and T_3 concentrations in the nuclear extract and the α -GPD activity remained elevated. Whereas the corrected values for K_a were unchanged, the corrected values for C_{max} tended to decrease compared with those in the rats given the same doses of T_4 alone (Table 2).

Discussion

The present studies with IOP indicate that T_4 itself increases the mitochondrial α -GPD activity and the DNA content of rat liver, and further, T_4 is bound to the nuclear receptor after the long-term (7-day) administration. These results strongly suggest that T_4 interacts with the nuclear receptor to exert the hormone action.

Since T_4 is quickly converted into T_3 in peripheral tissues, it is difficult to distinguish the hormonal effects of T_4 , if any, from those of T_3 without the aid of a conversion inhibitor. Although 6-n-propylthiouracil was used previously to examine the hormonal activity of T_4 itself, the results obtained were not conclusive because of its incomplete (60%) inhibition of the conversion (Oppenheimer *et al.*, 1972a; Frumess & Larsen, 1975). In the first series of experiments, the administration of IOP to the rats given 2.4 μg of T_4 successfully blocked the conversion into T_3 . The serum T_3 concentration was

decreased to the hypothyroid level, and no T_3 was detected in the nuclear extract. Consequently, the observed increases in the mitochondrial α -GPD activity from 17.8×10^{-3} to 49.2×10^{-3} $\Delta A_{500}/\text{min}$ per mg of protein and in the hepatic DNA content could only be ascribed to T_4 . Conceivably, these hormonal effects may have been induced by a small amount of T_3 contaminating the injected T_4 preparation or converted from the injected T_4 . However, this appears unlikely, because no T_3 was demonstrated in the nuclear extract.

It has been reported that T_4 is 3–8 times less potent than T_3 , depending upon the assays employed to assess the hormonal activities (Jorgensen, 1978; Koerner *et al.*, 1975). In the present study the hormonal activity of T_4 was 5.4 times weaker than that of T_3 on the basis of the mitochondrial α -GPD activity determined. The α -GPD activity reaches a maximum 4 days after injection of T_3 (Oppenheimer *et al.*, 1978). The activity averaged 190.8×10^{-3} $\Delta A/\text{min}$ per mg of protein after injection of 2 μg of T_3 for 2 weeks (Nakamura *et al.*, 1979a) and 49.2×10^{-3} $\Delta A/\text{min}$ per mg of protein after injection of equimolar T_4 + IOP for 1 week. Given that the α -GPD activities for the hypothyroid controls were 19.9 (Nakamura *et al.*, 1979a) and 17.8 $\Delta A/\text{min}$ per mg of protein in separate experiments, the ratio of the increased α -GPD activity (E) of rats given T_3 rather than T_4 ($E_{2\mu\text{g of } T_3} - E_{\text{hypo}})/(E_{2.4\mu\text{g of } T_4 + \text{IOP}} - E_{\text{hypo}})$ is calculated to be 5.4 [$(190.8 - 19.9)/(49.2 - 17.8) = 5.44$]. If one uses the value of 78.9×10^{-3} $\Delta A/\text{min}$ per mg protein obtained after the administration of 2.4 μg of T_4 alone, the ratio would be inaccurately low (2.8) [$(190.8 - 19.9)/(78.9 - 17.8) = 2.80$] owing to the generation of T_3 from T_4 .

In contrast with the induction of α -GPD activity, T_4 increased the DNA content of the liver, despite the absence of nuclear T_3 . The latter effect required 7 days after T_4 injection, presumably because of the time required for cell proliferation. It is unclear why T_4 is as potent as T_3 in this respect. Growth is known to be more sensitive to T_4 than the calorogenic response (Evans *et al.*, 1960). It seems likely that T_4 is sufficiently active to promote cell proliferation, though it is less potent than T_3 .

The present finding, that T_4 has intrinsic hormonal activity, is consistent with previous reports. Samuels *et al.* (1979) found that T_4 induced a 3-fold increase in the growth rate of GH₁ cells, a rat pituitary tumour cell line, when bound to the nuclei. More recently Galton & Cohen (1980) have reported that, in pre-metamorphic tadpoles, T_4 plays a more important hormonal role than T_3 . Conversely, Larsen *et al.* (1979) and Obregon *et al.* (1980) suggested that T_4 possessed no intrinsic activity in rat pituitary thyrotrophs, since T_4 could not suppress serum thyrotropin when its conversion into T_3 was blocked by IOP. Most likely this paradox

was due to inherent differences among the tissues examined. Frumess & Larsen (1975) have reported that 6-n-propylthiouracil disproportionately altered the serum thyrotropin, hepatic α -GPD activity and growth rate. They could not exclude the possibility that this phenomenon resulted from relatively different sensitivities of these tissues to T_3 and T_4 . The present study clearly indicates that T_4 is an active hormone, yet is also a prohormone. This situation is analogous to that of testosterone in that testosterone is known to be an active hormone, and is also converted into dihydrotestosterone, which is twice as potent (Paulsen, 1974).

Surprisingly T_4 was present in the liver nuclear extract at a concentration comparable with that of T_3 . It is unlikely that the T_4 was non-specifically bound to the outer surface of the nuclei, because no T_4 was demonstrated in the nuclear extract obtained from the rats given 48 or 480 μ g of T_4 for 3 days. Although the binding site for T_4 in the nuclear extract is not fully characterized, it seems reasonable to conclude that T_4 is bound to acidic NHP. Previous studies indicated that T_4 binding to the liver nuclear receptor was 10–15% of the total nuclear iodothyronine in intact animals (Surks & Oppenheimer, 1977), and suggested that it may be non-specific binding (Oppenheimer *et al.*, 1972b; Surks *et al.*, 1973). These reports are therefore not in agreement with our data. It is noteworthy that the NHP-bound T_4 was demonstrable only when T_4 was administered either for 7 days or together with IOP for 3–7 days. This observation suggests that the failure to detect T_4 binding to the nuclear NHP in the previous studies is probably due to insufficient exposure of NHP to T_4 and that the T_4 -NHP complex may have been formed as a result of a long-term interaction between T_4 and the nuclear NHP.

It was noted that the $C_{\max}$ and K_a of the nuclear T_3 receptor were significantly decreased after the administration of 2.4 μ g of T_4 + IOP for 7 days. These effects may have been due to a toxic effect of the administered IOP or the resultant decrease in food intake, because the liver nuclear receptor is known to decrease in starvation (Burman *et al.*, 1977; Schussler & Orlando, 1978) and partial hepatectomy (Dillmann *et al.*, 1978).

In the rats given 48 or 480 μ g of T_4 for 3 days, the nuclear extracts contained high concentrations of T_3 but not of T_4 . The $C_{\max}$ values of the nuclear receptors were markedly increased, whereas the K_a values were unchanged. The markedly increased α -GPD activities were ascribed to nuclear T_3 derived from T_4 conversion in peripheral tissues. These results are consistent with those obtained after the administration of T_3 (Hamada *et al.*, 1979; Nakamura *et al.*, 1979a,b), and agree with the popular current concept that T_4 is converted into T_3 ,

which in turn binds to the nuclear receptor (Ingbar & Braverman, 1975; Oppenheimer *et al.*, 1972b, 1976; Chopra *et al.*, 1978).

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