Effects of Hypo- and Hyper-Thyroidism on Liver Composition, Blood Glucose, Ketone Bodies and Insulin in the Male Rat

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1. Thyroidectomized rats injected daily with 0, 0.1, 2 or 25 μg of L-thyroxine/100g body wt. were compared with intact controls. In plasma, the protein-bound iodine was decreased in the rats given the 0 or 0.1 μg doses and increased in those given the 25 μg dose. 2. Blood glucose decreased in those given 2 μg and was augmented in those given 25 μg, and ketone bodies were the same in all the groups. 3. Plasma insulin was lowest in the rats given the 0 or 0.1 μg doses and was highest in those given the 2 or 25 μg doses of thyroxine. 4. After 48h starvation, the decrease in blood glucose and increase in ketone bodies observed in all the groups was greatest in the group not supplemented with thyroxine. 5. Plasma insulin concentrations remained at the value for fed animals in the rats given the 25 μg dose of thyroxine but decreased in the other groups. 6. In fed animals, concentrations of hepatic DNA P, citrate, total fatty acids and acetyl-CoA were similar in all the groups, and glycogen was low only in the rats given the 25 μg dose of thyroxine. 7. After 48h starvation, liver DNA P, total fatty acids and acetyl-CoA increased in all the groups, except in the rats given the 25 μg dose, where both total fatty acids and acetyl-CoA remained at the value for fed animals. Liver citrate did not change in the groups given the 0 or 25 μg doses of thyroxine, but decreased in the other groups. 8. The results are discussed in relation to the regulation of intermediary metabolism in hypo- and hyper-thyroidism.

The influence of thyroid hormones on intermediary metabolism is not completely understood. When the supply of thyroid hormones to the tissues is inadequate, oxidative processes, energy expenditure and thermogenesis are decreased. In most instances, the turnover rates of metabolites are slower, and final steady-state concentrations depend on the establishment of a new equilibrium between decreased anabolism and diminished catabolism, as has been reviewed by Metzger & Freinkel (1971). Plasma concentrations of free fatty acids after short periods of starvation in hypothyroid subjects are low or normal (Hamburger *et al.*, 1963; Harlan *et al.*, 1963). In hypothyroidism there is a decrease in gluconeogenesis (Menahan & Wieland, 1969) and a diminished glucose utilization in some tissues (Shames *et al.*, 1968), although in others, such as in adipose tissue, glucose utilization does not seem to differ from that in normal subjects (Bray & Goodman, 1968), and concentrations of liver glycogen and blood glucose are normal in the fed state or after a short period of starvation (Lamberg, 1965; Metzger & Freinkel, 1971).

Hyperthyroidism is also associated with alterations in the metabolic interrelationships between carbohydrate and fat metabolism (Freinkel & Metzger, 1971). It has been demonstrated that there is an enhanced turnover of stored lipids (Bressler & Wittels, 1966; Frey, 1967), glucose synthesis (Freedland & Krebs, 1967; Menahan & Wieland, 1969) and glucose utilization (Rabinowitz & Myerson, 1967). Thus hepatic gluconeogenesis, ketogenesis and lipogenesis are the most altered pathways during changes in thyroid function. These pathways are possibly influenced by the regulatory effects that the content of acetyl-CoA and citrate (Newsholme & Gevers, 1967; Lowenstein, 1968) can exert on certain enzymes. The steady-state concentration of some of these metabolites in the starved rat has been evaluated in an attempt to understand the regulation *in vivo* of the above-mentioned pathways when food is withheld (Herrera & Freinkel, 1968). It is the purpose of the present work to investigate the effect of changes in thyroid function on these parameters and on liver composition in fed and 48h-starved rats.

Materials and Methods

Animals

Young male Wistar rats weighing 50–60g were fed on a medium-residue, low-iodine diet (0.04–0.09 μg of iodine/g) (Escobar del Rey *et al.*, 1968), surgically

thyroidectomized (Zarrow et al., 1964) and injected daily intraperitoneally thereafter with 0, 0.1, 2 or 25 μg of L-thyroxine/100g body wt. for 45–61 days, after which they were killed. They were compared with age-matched intact male controls under the same diet supplemented with 1.7 μg of KIO_3/g and injected daily with 0.9% NaCl during the same period of time.

Animals were killed by decapitation and without anaesthesia between 10a.m. and noon. Blood was collected into heparinized chilled beakers and a piece of liver was placed in liquid N_2 in less than 10s from decapitation.

Blood components

Immediately after collection, a sample of blood was placed in ice-cold water, deproteinized with $Ba(OH)_2 - ZnSO_4$ (Somogyi, 1945) and analysed for glucose (Huggett & Nixon, 1957) and total ketone bodies (Bessman & Anderson, 1957). Immuno-reactive insulin was measured in the plasma by a double-antibody technique (Hales & Randle, 1963) by using a radioactive insulin kit obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Rat insulin (kindly given by Novo Industries, Copenhagen, Denmark) was used as standard. Plasma was pooled for each group and frozen for determination of the protein-bound iodine by a modified Zak procedure (Benotti & Benotti, 1963).

Analysis of liver

Portions of the frozen liver were analysed for protein by the procedure of Lowry et al. (1951) after alkali digestion, with bovine serum albumin as standard. Separately weighed portions of frozen liver were digested with KOH for precipitation of glycogen with ethanol (Good et al., 1933). The precipitates were solubilized in water and reprecipitated twice with ethanol for purification before acid hydrolysis (2.5M-H_2SO_4; 2h; 100°C) and were then assayed enzymically with glucose oxidase (Huggett & Nixon, 1957). Another portion of frozen liver was extracted with 20vol. of chloroform–methanol (2:1, v/v). The lipid extracts were purified by the method of Folch et al. (1957). One sample was used to determine lipid P (Fiske & Subbarow, 1925) after digestion with 72% (v/v) $HClO_4$ as described by Freinkel (1958). A second sample was evaporated to dryness under N_2 at 45°C, saponified with 1M-KOH in 95% (v/v) ethanol for 2h at 100°C and the total fatty acids were extracted into heptane after acidification. Final heptane extracts were evaporated to dryness under N_2 at 45°C and resuspended in chloroform for determination of fatty acids by the method of Duncombe (1963). DNA P was isolated from the residual pellet after lipid extraction (Schmidt &

Thannhauser, 1945); inorganic P was determined (Fiske & Subbarow, 1925) after digestion with 72% $HClO_4$.

Acetyl-CoA was determined in the extract of another sample of the frozen liver with an Eppendorf recording fluorimeter (Herrera & Freinkel, 1967). Another sample from the same tissue extract as that used for the determination of acetyl-CoA was used for measurement of citrate (Moellering & Gruber, 1966).

Results

Circulating components in rats of different thyroidal status

When rats were thyroidectomized at the end of weaning, and fed on a low-iodine diet for 45–61 days until they were killed, they showed a great decrease in plasma protein-bound iodine concentration when compared with their age-matched intact controls on the same diet (Table 1). The daily injection of thyroxine (0.1 μg) administered to the thyroidectomized animals doubled their plasma protein-bound iodine and, although statistical analysis is not possible because pooled samples were used, this parameter remained lower than in the controls. The difference from the controls was smaller when the thyroidectomized rats were treated with 2 μg of thyroxine (Table 1). The injection of 25 μg of thyroxine into thyroidectomized rats produced a rise in plasma protein-bound insulin up to values more than twice that in the controls (Table 1). Starvation for 48h altered the absolute values for most parameters, but the relative differences between the groups remained the same as in the fed state (Table 1).

When the animals were fed, blood glucose was the same in the thyroidectomized rats treated with either 0 or 0.1 μg of thyroxine, lower in animals treated with 2 μg and higher in those treated with 25 μg than in the intact controls (Table 1). After 48h of starvation, blood glucose concentration fell in all the groups, but the greatest fall was observed in the thyroidectomized rats treated with 0 or 0.1 μg of thyroxine and the smallest fall occurred in those rats treated with 25 μg of thyroxine, the differences from the controls being statistically significant (Table 1).

Blood ketone-body concentrations in the fed animals were similar in all the groups (Table 1). Starvation made this parameter increase in all groups, but the value obtained in the thyroidectomized animals, not treated with thyroxine, was higher ($P < 0.05$) than in the corresponding controls, whereas the other groups did not show significant differences from the intact animals (Table 1).

Plasma concentrations of insulin in the fed, thyroidectomized rats treated with either 0 or 0.1 μg of thyroxine were lower than in the controls ($P < 0.02$)

Table 1. *Effect of thyroidectomy and treatment with L-thyroxine on blood components in the rat*

Rats were thyroidectomized after weaning and injected daily intraperitoneally with different doses of L-thyroxine for 45–61 days. They were then killed by decapitation. The results are given as means ±S.E.M. with the number of animals in each group shown in parentheses, except where analyses were made on a pool of samples from all the animals in the group. P refers to the differences between fed and 48h-starved groups and P' to the difference between each group and its respective control under the same dietary status (N.S., not significant, i.e. P or $P' > 0.05$). Other details are given in the Materials and Methods section.

Group (μg of L-thyroxine/100g body wt.)	Dietary status	Plasma protein-bound iodine (μg/100ml)	Blood glucose (mg/100ml)	P'	Blood ketone bodies (μmol/ml)	P'	Plasma insulin (μunits/ml)	P'
Intact controls (0)	Fed	6.9 (11)	104±2 (10)		0.4 (11)		59±6 (11)	
	48h-starved	3.2 (11)	68±4 (11)		2.0±0.2 (13)		34±3 (11)	
			$P<0.001$				$P<0.001$	
Thyroidectomized (0)	Fed	0.3 (8)	96±4 (9)	N.S.	0.5 (9)		35±7 (8)	<0.02
	48h-starved	0.4 (6)	47±4 (11)	<0.001	2.7±0.2 (13)	<0.05	23±3 (6)	<0.05
			$P<0.001$				N.S.	
Thyroidectomized (0.1)	Fed	0.6 (13)	94±4 (12)	N.S.	0.6 (12)		42±3 (13)	<0.02
	48h-starved	1.1 (13)	59±2 (13)	<0.05	2.5±0.3 (15)	N.S.	27±2 (13)	N.S.
			$P<0.001$				$P<0.001$	
Thyroidectomized (2)	Fed	5.9 (9)	97±2 (9)	<0.05	0.7 (9)		86±11 (9)	<0.05
	48h-starved	6.7 (6)	68±3 (5)	N.S.	2.4±0.2 (7)	N.S.	35±5 (6)	N.S.
			$P<0.001$				$P<0.01$	
Thyroidectomized (25)	Fed	13.4 (8)	116±4 (8)	<0.05	0.4 (8)		103±15 (8)	<0.01
	48h-starved	15.6 (8)	90±4 (7)	<0.01	1.5±0.2 (7)	N.S.	80±17 (8)	<0.01
			$P<0.001$				N.S.	

and this would agree with the decreased secretion of insulin in hypothyroid rats reported by Malaisse *et al.* (1967). However, when the thyroidectomized rats were treated with $2\mu g$ of thyroxine they showed higher concentrations of plasma insulin than did the controls ($P<0.05$), and this difference was even greater when they were treated with $25\mu g$ of thyroxine ($P<0.01$). After 48h starvation the plasma insulin concentration fell in all groups when compared with fed rats, except in the thyroidectomized rats treated with $25\mu g$ of thyroxine, where it did not change significantly (Table 1). On comparison with the starved controls, the plasma insulin concentration of thyroidectomized rats was lower ($P<0.001$); when they were treated with 0.1 or $2\mu g$ of thyroxine it remained the same, and it was higher in those animals injected with $25\mu g$ ($P<0.01$).

Effect of thyroidectomy and treatment with L-thyroxine on the body and liver weights and liver composition

Results are summarized in Table 2. Although before thyroidectomy there were no differences in the body weight of the rats in the different groups, the weight of the thyroidectomized animals was 59.2% lower than that of the intact controls ($P<0.05$) at the time the animals were killed. The daily injection of $0.1\mu g$ of thyroxine/100g body wt. was sufficient to restore some growth capability. The weight became equal to that of the controls when the thyroidectomized rats were treated with $2\mu g$ of thyroxine/100g body wt. (Table 2). However, treatment of the thyroidectomized animals with $25\mu g$ of thyroxine/100g body wt. caused a diminution of body weight

compared with the controls. Starvation for 48h caused the body weight to fall in all the groups ($P<0.001$), but the relative differences between the groups remained as in the fed groups. These changes with starvation are not shown in Table 2 because the fed and starved animals were not taken from the same groups of rats.

Parallel changes to the body weight are found for the absolute liver weight and the liver weight expressed per 100g body wt. in the thyroidectomized rats treated with 0, 0.1 or $2\mu g$ of thyroxine, compared with their intact controls (Table 2). Liver weights of the thyroidectomized animals injected with $25\mu g$ of thyroxine are the same for fed animals, and greater for starved animals than their respective controls. When the liver weights are expressed per 100g body wt., the thyroidectomized animals injected with $25\mu g$ of thyroxine have bigger livers than their controls in either dietary condition (Table 2). These differences in liver weight prompted a study of DNA P as an index of cellularity (Table 2). The concentration of DNA P was the same in all the groups when fed. After 48h starvation the concentration of DNA P rose in all the groups, and the rise was most pronounced in the thyroidectomized rats not treated with thyroxine. As on other occasions (Herrera & Freinkel, 1968; Herrera *et al.*, 1969) the total amount of DNA P in the whole liver was found to be the same in starved as in fed animals, suggesting that there was no change in the total number of cells. Phospholipid P was not different in the fed thyroidectomized rats treated with 0, 0.1 or $2\mu g$ of thyroxine compared with their controls, but increased in those rats injected with $25\mu g$ of thyroxine. After

Table 2. *Effect of thyroidectomy and treatment with L-thyroxine*

Rats were thyroidectomized after weaning and injected daily intraperitoneally with different doses of L-thyroxine for 45–61 days. with the number of animals in each group in parentheses. P refers to the differences between fed and 48h-starved groups and P' ... P or $P'>0.05$). Other details are given in the Materials and Methods section.

Group (μg of L-thyroxine/100g body wt.)	Dietary status	Body wt. (g)	P'	Liver wt. (g)	P'	Liver wt. (g/100g body wt.)	P'
Intact controls (0)	Fed	237 ± 11 (9)		10.9 ± 0.7 (9)		4.6 ± 0.2 (9)	
	48h-starved	241 ± 16 (6) N.S.		6.9 ± 0.4 (7) $P<0.001$		2.8 ± 0.0 (6) $P<0.001$	
Thyroidectomized (0)	Fed	97 ± 6 (9)	<0.001	3.5 ± 0.2 (9)	<0.001	3.5 ± 0.2 (7)	<0.001
	48h-starved	84 ± 3 (7) N.S.	<0.001	2.1 ± 0.1 (7) $P<0.001$	<0.001	2.6 ± 0.1 (7) $P<0.001$	<0.02
Thyroidectomized (0.1)	Fed	163 ± 8 (7)	<0.001	6.1 ± 0.5 (7)	<0.001	3.8 ± 0.3 (7)	<0.05
	48h-starved	159 ± 4 (7) N.S.	<0.001	3.7 ± 0.1 (7) $P<0.001$	<0.001	2.4 ± 0.1 (7) $P<0.001$	<0.001
Thyroidectomized (2)	Fed	244 ± 21 (6)	N.S.	11.0 ± 0.5 (6)	N.S.	5.0 ± 0.4 (6)	N.S.
	48h-starved	237 ± 13 (5) N.S.	N.S.	7.1 ± 0.4 (5) $P<0.001$	N.S.	3.1 ± 0.3 (5) $P<0.01$	N.S.
Thyroidectomized (25)	Fed	192 ± 9 (8)	<0.01	10.9 ± 0.2 (7)	N.S.	5.4 ± 0.3 (7)	<0.05
	48h-starved	198 ± 12 (7) N.S.	<0.05	8.5 ± 0.6 (7) $P<0.001$	<0.05	4.3 ± 0.1 (7) $P<0.01$	<0.001

48h of starvation, the thyroidectomized rats treated with 0.1 or $2\mu g$ of thyroxine, as well as their controls, showed an increase in the phospholipid P, whereas in those animals treated with either 0 or $25\mu g$ of thyroxine there was no change on starvation.

Total fatty acids and proteins per g of liver were the same in all the fed groups (Table 2). Starvation produced a significant increase in both parameters in the liver of all the animals except in those thyroidectomized and treated with $25\mu g$ of thyroxine, where the difference from the fed animals from the same group was not significant (Table 2). The percentage of liver glycogen was not different in the fed thyroidectomized rats treated with 0, 0.1 or $2\mu g$ of thyroxine or in the intact controls, whereas in the thyroidectomized rats injected with $25\mu g$ of thyroxine it was significantly lower than in the controls (Table 2). Starvation produced a decrease in the percentage of liver glycogen in all the groups, but in the thyroidectomized rats it was lower in those treated with 0 or $0.1\mu g$ of thyroxine and it remained the same in those injected with 2 or $25\mu g$ of thyroxine (Table 2).

Effect of thyroidectomy and treatment with thyroxine on liver acetyl-CoA and citrate

Acetyl-CoA concentration in the liver of all the animals of different thyroid status was the same when the rats were fed (Table 3). Starvation produced an increase in the liver steady state of this metabolite in the thyroidectomized rats treated with 0, 0.1 or $2\mu g$ of thyroxine, as in the controls, whereas in those treated with $25\mu g$ of thyroxine it did not change. In the fed animals citrate content per g of liver was the

same in all the situations studied (Table 3); here again, starvation emphasized differences among the groups: the concentration of citrate in the liver of the thyroidectomized rats treated with either 0.1 or $2\mu g$ of thyroxine fell, as it did in the controls, whereas the value was not significantly altered with starvation in the thyroidectomized animals treated with 0 or $25\mu g$ of thyroxine (Table 3).

Discussion

The present study shows that differences in the thyroid status of the animals greatly altered their carbohydrate–lipid interrelationships, alterations that are especially apparent when food was withheld.

Interrelationships between carbohydrate and lipid metabolism in thyroidectomized rats

Thyroidectomized rats fed *ad libitum* on a diet of low iodine content showed normal concentrations of most of the metabolites studied. A diminished liver gluconeogenesis in fed hypothyroid rats has not previously been described. We have shown that the steady-state concentration of regulatory metabolites such as acetyl-CoA and citrate are the same in the thyroidectomized rats as in the intact controls. The low concentration of circulating insulin (Malaisse *et al.*, 1967) might also be contributing to the maintenance of gluconeogenesis in these animals at the normal rate. This equilibrium was broken when food was withheld for 48h. Both blood glucose and liver glycogen declined to minimal values. An increase in the urea production by the perfused liver of these animals

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on the body and liver weights and liver composition in the rat

They were then killed by decapitation. Pieces of liver were frozen within 10s in liquid N_2. The results are given as means$\pm$S.E.M. to the differences between each group and its respective control under the same dietary status (N.S., not significant, i.e.

Liver DNA P ($\mu g/g$)	P'	Liver phospholipid P (mg/g)	P'	Liver total fatty acids ($\mu mol/g$)	P'	Liver proteins (mg/g)	P'	Liver glycogen (%)	P'
181±6 (8)		1.14±0.02 (7)		152±8 (8)		138±3 (8)		5.1±0.2 (6)	
258±11 (7)		1.39±0.04 (7)		210±24 (7)		161±3 (7)		0.4±0.1 (7)	
P<0.001		P<0.001		P<0.05		P<0.001		P<0.001	
191±13 (7)	N.S.	1.17±0.04 (7)	N.S.	150±7 (7)	N.S.	150±4 (6)	<0.02	3.7±0.7 (6)	N.S.
327±22 (7)	<0.02	1.17±0.07 (7)	<0.02	231±13 (6)	N.S.	170±6 (8)	N.S.	0.0±0.0 (6)	<0.02
P<0.001		N.S.		P<0.001		P<0.05		P<0.001	
188±24 (7)	N.S.	1.03±0.04 (6)	N.S.	145±11 (6)	N.S.	135±7 (7)	N.S.	5.8±0.6 (6)	N.S.
270±12 (7)	N.S.	1.45±0.03 (6)	N.S.	270±33 (7)	N.S.	164±4 (7)	N.S.	0.1±0.0 (7)	<0.05
P<0.01		P<0.001		P<0.01		P<0.001		P<0.001	
178±12 (5)	N.S.	1.19±0.03 (5)	N.S.	144±5 (6)	N.S.	134±3 (6)	N.S.	4.4±0.1 (4)	N.S.
270±6 (4)	N.S.	1.43±0.03 (4)	N.S.	249±21 (5)	N.S.	157±4 (5)	N.S.	0.4±0.1 (5)	N.S.
P<0.001		P<0.001		P<0.001		P<0.01		P<0.001	
184±5 (6)	N.S.	1.33±0.06 (6)	<0.01	165±6 (5)	N.S.	153±4 (6)	<0.01	1.2±0.3 (6)	<0.001
283±9 (5)	N.S.	1.42±0.06 (5)	N.S.	209±27 (5)	N.S.	158±5 (5)	N.S.	0.2±0.0 (5)	N.S.
P<0.001		N.S.		N.S.		N.S.		P<0.01	

Table 3. *Effect of thyroidectomy and treatment with L-thyroxine on the steady-state concentrations of acetyl-CoA and citrate in the liver of the rat*

Rats were thyroidectomized after weaning and injected daily intraperitoneally with different doses of L-thyroxine during 45–61 days. They were then killed by decapitation. Pieces of liver were frozen within 10s in liquid N_2. The results are given as means±S.E.M. with the number of animals in each group shown in parentheses. *P* refers to the differences between fed and 48h-starved groups and *P'* to the differences between each group and its respective control under the same dietary status (N.S., not significant, i.e. *P* or *P'* > 0.05). Other details are given in the Materials and Methods section.

Group (μg of L-thyroxine/100g body wt.)	Dietary status	Acetyl-CoA (nmol/g)	*P'*	Citrate (nmol/g)	*P'*
Intact controls (0)	Fed	54±7 (7)		265±18 (8)	
	48h-starved	81±8 (6)		145±11 (6)	
		P<0.05		*P*<0.001	
Thyroidectomized (0)	Fed	62±4 (8)	N.S.	231±19 (8)	N.S.
	48h-starved	104±11 (7)	N.S.	222±33 (6)	N.S.
		P<0.01		N.S.	
Thyroidectomized (0.1)	Fed	50±6 (7)	N.S.	246±32 (7)	N.S.
	48h-starved	86±8 (6)	N.S.	147±16 (4)	N.S.
		P<0.01		*P*<0.05	
Thyroidectomized (2)	Fed	54±2 (5)	N.S.	244±22 (6)	N.S.
	48h-starved	94±11 (5)	N.S.	148±23 (4)	N.S.
		P<0.01		*P*<0.05	
Thyroidectomized (25)	Fed	61±6 (8)	N.S.	316±39 (7)	N.S.
	48h-starved	66±5 (7)	N.S.	381±6 (6)	<0.01
		N.S.		N.S.	

(Menahan & Wieland, 1969) would suggest a preservation rather than a decrease of liver gluconeogenesis. The activity of some gluconeogenic enzymes is decreased in hypothyroid rats (Menahan & Wieland, 1969), but the steady-state concentrations of regulatory metabolites for gluconeogenesis are maintained at the normal values. The concentration of phospholipids in the liver of the starved thyroidectomized rats is lower than in the controls, whereas that of their total fatty acids is the same, which suggests an increased concentration of free and esterified fatty acids of neutral lipids in these animals. This abundance of fatty acids in the liver of the hypothyroid animals when starved is not surprising, because it is known that fat retention is favoured in these animals (Scow, 1951). The adequate availability of lipids in these animals when starved may allow them to have sufficient acetyl-CoA in their livers to maintain citrate at the same concentration as in the fed animal, to have high concentrations of circulating ketone bodies and to maintain a steady-state concentration of acetyl-CoA in their livers the same as in the intact controls. The preservation of liver citrate with starvation in the hypothyroid rats deserves special attention. In normal animals citrate concentrations fall, as we have shown here and in other situations (Herrera & Freinkel, 1968). This could be explained by a lack of oxaloacetate, which

is being used for gluconeogenesis and would direct acetyl-CoA from the tricarboxylic acid cycle to ketone-body formation (Krebs, 1966). According to this explanation, our results suggest that oxaloacetate is not lacking in the thyroidectomized rats when starved.

Effect of small doses of thyroxine on intermediary metabolism in thyroidectomized rats

The administration of 0.1 μg of thyroxine/100g body wt. to the thyroidectomized rats was sufficient to normalize most of the metabolic parameters studied in both fed and starved animals, despite the fact that the plasma protein-bound iodine was between 66 and 91 % lower than the controls. These doses of thyroxine were about one-twentieth of those needed to depress high rates of pituitary release of thyroid-stimulating hormone to normal values (Purves, 1964). The possibility exists that maintenance of normal intermediary metabolism requires extremely small amounts of thyroid hormones. Indeed, alterations of intermediary metabolism of intensely hypothyroid rats may well be secondary to the inadequate functioning of the adenohypophysis and the pituitary-dependent glands of such animals. On the other hand, the fact that not all the concentrations of metabolites studied are normal in the thyroidectomized rats

injected with 0.1 μg of thyroxine would suggest a different sensitivity of the pathways in which they are involved to the amount of thyroid hormone available.

Metabolic alterations in hyperthyroidism

The daily injection of 25 μg of thyroxine/100 g body wt. was sufficient to make the thyroidectomized rats hyperthyroid, as shown by their high plasma concentrations of protein-bound iodine. The main metabolic manifestation that is apparent in these animals is their decreased body weight, probably produced by the net catabolism that prevails when the amount of thyroid hormones available to the peripheral tissues is supranormal (Freinkel & Metzger, 1971). In the fed state the only parameters studied that were altered were the concentrations of liver glycogen and circulating glucose and insulin.

Liver glycogen was diminished in the hyperthyroid rats, confirming the results of others (Coggeshall & Greene, 1933; Kuriyama, 1918), and could reflect augmented glycogenolysis as a consequence of increased adenylate cyclase activity as well as by potentiation of the response to the catecholamines, as reviewed by Freinkel & Metzger (1971). Gluconeogenesis may also be enhanced in these thyroidectomized animals treated with 25 μg of thyroxine, because gluconeogenic enzymes are augmented after thyroxine treatment (Szepesi & Freedland, 1969; Young, 1968; Murad & Freedland, 1967; Menahan & Wieland, 1969) and perfused livers from hyperthyroid rats exhibit increased rates of glucose formation from different substrates (Freedland & Krebs, 1967; Menahan & Wieland, 1969). Although glucose oxidation and removal might be increased in hyperthyroidism (Rabinowitz & Myerson, 1967), our thyroidectomized rats treated with 25 μg of thyroxine are hyperglycaemic. It would appear that net glycogenolysis and gluconeogenesis rates exceeded glucose removal. This diabetogenic situation appeared together with high circulating concentrations of insulin, in contradiction to the results of Malaisse *et al.* (1967), who showed that treatment of normal rats with high doses of thyroxine caused marked decreases in both pancreatic content and secretion of insulin. These authors utilized approximately ten times the dose that we used and different experimental conditions. It is possible that the thyroxine may have a biphasic effect on insulin metabolism, depending on the dose used. That high concentrations of circulating insulin in our hyperthyroid animals are due to increased secretion and not to decreased degradation is supported by the fact that accelerated degradation of insulin has been reported in thyrotoxic animals (Cohen, 1957; Elgee & Williams, 1955). The diabetic metabolic situation in our hyperthyroid animals, together with their high circulating con-

centrations of insulin, would suggest an increased resistance to this hormone.

As diversion to fat is exaggerated and accelerated in thyrotoxicosis even in the fed state, as reviewed by Freinkel & Metzger (1971), the lipid reserves are probably depleted in the hyperthyroid rats after 48 h of starvation. This is supported by the finding of a lower concentration of blood ketone bodies in these animals. The lack of lipid reserve seems to be partially compensated by an increased catabolism of proteins, as suggested by the greater decrease in the total content of protein in the liver. The metabolic state is comparable with other diabetic situations, as in obese–hyperglycaemic mice, where regulatory metabolites in the liver and gluconeogenesis do not change with starvation, but where hyperglycaemia and high circulating concentrations of insulin are maintained (Sandler *et al.*, 1968; Stauffacher *et al.*, 1967).

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