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EFFECTS OF GLUCOSE, INSULIN AND ADRENALIN ON GLYCEROL METABOLISM IN ADIPOSE TISSUE FROM HYPOTHYROID RATS *

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The in vitro metabolism of glycerol by epididymal fat pads from thyroidectomized rats daily injected with either 0, 0.1 or 1.8 μg of L-thyroxine/100 g body wt. was compared with that from intact controls. The basal as well as the adrenalin- or glucose-enhanced release of glycerol to the medium were similar in the tissues from all the groups. The effect of insulin in decreasing the lipolytic action of adrenalin was greater in the thyroidectomized animals treated with either 0 or 0.1 μg of thyroxine than in the other two groups. The utilization of [1-^{14}C]glycerol for the formation of CO_2 and glyceride glycerol was increased in the thyroidectomized rats; this effect was smaller when the animals were treated with 0.1 μg of thyroxine and disappeared when they were treated with 1.8 μg. In the presence of glucose the difference in utilization of glycerol between the groups disappeared. The formation of fatty acids from glycerol was greater in the presence than in the absence of glucose and was similar in the hypothyroid animals and the controls. The effect of adrenalin in decreasing the utilization of [1-^{14}C]glycerol was less in the tissues from hypothyroid rats than in the controls. The decrease of the action of adrenalin by insulin in the tissues from thyroidectomized rats treated with 0 or 0.1 μg of thyroxine was greater than in the controls. The increased capacity to form glyceride glycerol from glycerol in tissues from hypothyroid animals contributes to the high re-esterification of fatty acids described for these animals. The effect of glucose is explained in terms of its competition with glycerol for the synthesis of α-glycerophosphate.

Keywords: thyroidectomy; lipogenesis; epididymal fat-pad.

Hypothyroidism is generally associated with a decreased lipolytic activity in adipose tissue and a decreased response of this tissue to adrenalin stimulation (Debons and Schwartz, 1962; Deykin and Vaughan, 1963; Fisher and Ball, 1967). However, there is evidence of a normal response to adrenalin in adipose tissue from thyroidectomized animals (Rosenfeld and Rosenberg, 1965; Goodman and Bray, 1966). There is also general agreement that re-esterification of unesterified fatty acids in

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vitro is enhanced in adipose tissue from hypothyroid rats (Goodman and Bray, 1966; Fisher and Ball, 1967). We have previously observed that the rate of glycerol utilization is higher in adipose tissue from fasted thyroidectomized rats than from normal controls (Montoya and Herrera, 1974). Actually, when the net release of glycerol by the tissue is corrected for the higher glycerol metabolism, it appears that the rate of lipolysis is enhanced also in the adipose tissue from these fasted thyroidectomized animals. Since glycerol utilization by adipose tissue in vitro is highly influenced by glucose, insulin and adrenalin (Dominguez and Herrera, 1976a, b), we have studied to what extent these hormones affect glycerol metabolism in adipose tissue from fed thyroidectomized rats.

MATERIALS AND METHODS

Animals

Young male Wistar rats weighing 50–60 g were fed on a medium-residue, low-iodine diet (0.05–0.1 μg iodine/g; Escobar del Rey et al., 1968), consisting of: corn flour, 6 kg; NaCl, 0.15 kg; $CaCO_3$, 0.15 kg; wheat gluten, 2.5 kg and dried brewer's yeast, 1 kg. The rats were surgically thyroidectomized (Zarrow et al., 1964) and injected daily intraperitoneally with either 0.9% (w/v) NaCl, 0.1 μg L-thyroxine/100 g body wt. or 1.8 μg L-thyroxine/100 g body wt., for 35–40 days. These animals were compared with age-matched intact controls maintained 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. Rats were killed by decapitation and blood was collected into heparinized chilled beakers. The plasma was used for determining protein-bound iodine by a colorimetric procedure (Benotti and Benotti, 1963).

In vitro incubations

Pieces of epididymal fat-pad (14.4 ± 0.4 mg) were dissected immediately after sacrifice and placed in 20-ml vials containing 0.5 ml Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit et al., 1964), supplemented with bovine serum albumin (20 mg/ml) defatted by the method of Chen (1973). Fresh solutions of [1-^{14}C]glycerol (0.2 μCi, 109.65 μCi/mmol) with addition of glucose (10 mM), rat insulin (400 μU/ml; generously provided by Novo Industri A/S, Denmark), adrenalin (5.46 μM; adrenalin bitartrate; Sigma Co., St. Louis, Mo.) or insulin plus adrenalin in 0.5 ml of the buffer solution lacking albumin were pipetted into the vials. Then they were sealed, gassed for 5 min with O_2 + CO_2 (95 : 5) and incubated at 37°C in a Dubnoff metabolic shaker at 100 cycles/min. The incubation was stopped by addition of $HClO_4$ and CO_2 was trapped in hyamine 10-X hydroxide by gentle shaking at room temperature for 60 min (Herrera and Lamas, 1970; Herrera and Ayanz, 1972). The medium was neutralized with $KHCO_3$ and centrifuged at 4°C to remove $KClO_4$ and aliquots of the supernatant were taken for determination of glycerol (Garland and Randle, 1962). The incubated tissues were rinsed with 0.9%

NaCl and placed in chloroform–methanol for lipid extraction (Folch et al. 1957). The lipid extracts were washed once with 0.9% NaCl and three times with 1 M glycerol to remove the non-radioactive glycerol bound to the tissue. Portions of the lipid extracts were saponified in 1 M KOH in 95% (v/v) ethanol for 2 h at 100°C. Non-saponifiable lipids were extracted with heptane and fatty acids were extracted by a second wash with heptane after acidification with 1 M H_2SO_4. Radioactivity in the glyceride glycerol was calculated from the difference between radioactivities in the total lipids and in the heptane extracts. These procedures have been recently validated by recovery experiments (Dominguez and Herrera, 1976a). Deoxyribonucleic acid phosphorus (DNA-P) was isolated from the residual pellet after lipid extraction in portions of unincubated tissues (Schmidt and Tannhauser, 1945) and inorganic phosphorus was estimated (Fiske and Subbarow, 1925) after digestion with 72% $HClO_4$.

RESULTS

Although at the onset of the experiment there were no differences in body weight of the rats among the different groups, 35–40 days after surgery the thyroidectomized rats weighed 45% less than the intact controls (table 1). The daily injection of 0.1 μg L-thyroxine/100 g body wt. restored a considerable growth capability in the thyroidectomized animals but their body wt. did not reach the values of the controls except in the group treated with a daily dose of 1.8 μg/100 g body wt. (table 1). These changes were paralleled by those of the plasma protein-bound iodine concentration (table 1) which was very low in the thyroidectomized rats, slightly recuperated in the animals treated with 0.1 μg of L-thyroxine and completely normal in those treated with 1.8 μg, as compared with the intact controls. The weights of the epididymal fat pads differed among the groups in accordance with their body weights (table 1). The concentration of DNA-P per g of epididymal fat pad was higher in the thyroidectomized rats treated with either 0 or 0.1 μg of L-thyroxine than in the controls, but the differences were not statistically significant due to the variability of the values (table 1). The content of DNA-P per whole epididymal fat pad was actually decreased in the thyroidectomized rats not treated with L-thyroxine as compared to the controls, due to the smaller size of the tissue in those animals. (The DNA-P content per fat pad was, in fact, almost identical in all groups when corrected per 100 g body wt. (data not shown).)

The in vitro release of glycerol by pieces of epididymal fat pads incubated for 120 min is given in table 2. With the exception of a reduced release of glycerol by the tissues from the thyroidectomized rats injected with 0.1 μg of L-thyroxine, incubated in the presence of both insulin and adrenalin in the absence of glucose, the tissues from the thyroidectomized rats do not show any differences compared to the intact controls. The response to hormones and the presence of glucose in the medium was similar to that found previously in tissues from normal rats (Domin-

Table 1
Effect of thyroidectomy and treatment with L-thyroxine on body and epididymal fat pad weights and plasma protein-bound iodine concentration in the rat. Rats were thyroidectomized after weaning and injected daily (i.p.) with different doses of L-thyroxine for 35–40 days. They were killed by decapitation. The results are given as means ± S.E.M. with the number of animals in each group shown in parenthesis. *P* refers to the differences between each group and its control (N.S. = not significant; i.e. $P < 0.05$). Other details are given under Materials and Methods.

Group	Dose thyroxine (μg/100 g body wt.)	Body wt. (g)	Plasma protein-bound iodine (μg/100 ml)	Epididymal fat pads	
				Whole weight (g)	DNA-P (μg/g)
Intact controls	0	208 ± 6 (14)	4.79 ± 0.25 (12)	1.17 ± 0.10 (15)	369 ± 48 (8)
Thyroidectomized	0	113 ± 9 (17)	0.28 ± 0.08 (7)	0.52 ± 0.07 (12)	629 ± 178 (6)
P		<0.001	<0.001	<0.001	N.S.
Thyroidectomized	0.1	165 ± 6 (9)	0.81 ± 0.26 (7)	0.98 ± 0.11 (12)	475 ± 71 (7)
P		<0.001	<0.001	N.S.	N.S.
Thyroidectomized	1.8	205 ± 5 (16)	4.84 ± 0.70 (8)	1.31 ± 0.12 (10)	308 ± 29 (4)
P		N.S.	N.S.	N.S.	N.S.

Table 2

Effect of thyroidectomy and treatment with L-thyroxine on the in vitro production of glycerol by rat epididymal fat pads. Rats were thyroidectomized after weaning and injected daily with different doses of L-thyroxine for 35–40 days. They were killed by decapitation. Pieces of epididymal fat-pads were incubated in Krebs–Ringer bicarbonate medium containing albumin (10 mg/ml), [1-^{14}C]glycerol (0.2 μCi/ml, 109.65 μCi/mmol) and insulin (200 μU/ml) and/or adrenalin (2.73 μM) and/or glucose (5 mM). The results are given as means ± S.E.M. of 9–15 animals/group. *P* refers to the differences between each group and its respective control incubated under the same conditions and *P'* to the statistical differences between each value and that of the basal (no addition). Asterisks correspond to the difference between each group and the same group without addition of glucose (N.S. or no asterisks = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Group	Daily dose thyroxine (μg/100 g body wt.)	Glycerol release (μmol/100 mg tissue)						
		Basal	Insulin	*P'*	Adrenalin	*P'*	Ins + Adr	*P'*
(a) no glucose in the medium								
Intact controls	0	0.620 ± 0.13	0.507 ± 0.09	N.S.	1.31 ± 0.14	<0.01	0.985 ± 0.08	<0.05
Thyroidectomized	0	0.556 ± 0.15	0.752 ± 0.14	N.S.	1.14 ± 0.10	<0.01	0.873 ± 0.15	N.S.
P		N.S.	N.S.		N.S.		N.S.	
Thyroidectomized	0.1	0.439 ± 0.10	0.487 ± 0.08	N.S.	1.40 ± 0.42	N.S.	0.551 ± 0.10	N.S.
P		N.S.	N.S.		N.S.		<0.01	
Thyroidectomized	1.8	0.885 ± 0.27	0.632 ± 0.16	N.S.	1.44 ± 0.22	N.S.	1.30 ± 0.24	N.S.
P		N.S.	N.S.		N.S.		N.S.	
(b) 5 mM glucose in the medium								
Intact controls	0	0.925 ± 0.14	0.668 ± 0.11	N.S.	2.88 ± 0.38 **	<0.001	3.03 ± 0.47 ***	<0.001
Thyroidectomized	0	1.19 ± 0.20	1.13 ± 0.22	N.S.	3.32 ± 0.54 **	<0.001	2.92 ± 0.48 ***	<0.01
P		N.S.	N.S.		N.S.		N.S.	
Thyroidectomized	0.1	0.800 ± 0.13	0.839 ± 0.19	N.S.	3.36 ± 0.69 **	<0.01	2.27 ± 0.43 **	<0.01
P		N.S.	N.S.		N.S.		N.S.	
Thyroidectomized	1.8	1.39 ± 0.27	0.856 ± 0.22	N.S.	3.48 ± 0.76 *	<0.05	3.25 ± 0.49 **	<0.01
P		N.S.	N.S.		N.S.		N.S.	

guez and Herrera, 1976b) in all the groups: the effect of adrenalin enhancing the release of glycerol to the media was greater in the presence than in the absence of glucose, and although insulin alone did not modify the basal release of glycerol it reduced the effect of adrenalin when the medium was not supplemented with glucose (table 2).

The incubations of the epididymal fat pads were carried out in the presence of tracer amounts of [1-^{14}C]glycerol. The formation of $^{14}CO_2$ from this substrate by the tissues is shown in table 3. Although some of the differences are not significant due to the dispersion of the values, in all the conditions studied (i.e., in the presence or absence of either glucose, insulin or adrenalin) the tissues from the thyroidectomized rats not injected with L-thyroxine had greater conversion of glycerol to CO_2 than those from the intact controls (table 3). This difference is reduced when the thyroidectomized rats were injected with 0.1 μg of L-thyroxine, although the CO_2 values remained significantly higher than in the controls in the tissues incubated in the presence of both insulin and adrenalin (whether or not the medium was supplemented with glucose) and in those incubated in the presence of adrenalin in medium supplemented with glucose (table 3). The formation of $^{14}CO_2$ from labelled glycerol in the thyroidectomized rats treated with 1.8 μg L-thyroxine was the same as that found in the intact controls (table 3).

The synthesis of ^{14}C-labelled fatty acids from [1-^{14}C]glycerol was very small in the tissues incubated in the absence of glucose and was not affected by any of the conditions studied (table 4). As previously observed (Dominguez and Herrera, 1976b), the presence of glucose in the medium enhanced the formation of labelled fatty acids in all groups. Insulin increased this parameter in all groups, while adrenalin produced a significant decrease which was also similar in all groups (table 4). The presence of insulin in the media containing glucose diminished the adrenalin effect on the synthesis of fatty acids from glycerol and this effect of insulin was greater in the thyroidectomized rats treated with either 0 or 0.1 μg of L-thyroxine than in the other two groups (table 4).

The greatest effect of thyroidectomy was observed in the formation of ^{14}C-labelled glyceride glycerol from [1-^{14}C]glycerol. As shown in table 5, this parameter was intensely augmented in the tissues from thyroidectomized rats not injected with L-thyroxine in the absence of glucose. The effect was smaller in the tissues from thyroidectomized rats treated with 0.1 μg of thyroxine and completely disappeared in those treated with 1.8 μg. The presence of insulin in the medium did not affect this reaction while adrenalin produced a significant reduction in the formation of glyceride glycerol in the intact controls and the thyroidectomized rats treated with 1.8 μg of L-thyroxine but did not affect it in the tissues from thyroidectomized animals treated with either 0 or 0.1 μg of L-thyroxine. In a similar way, while insulin decreased the effect of adrenalin in the tissues from intact controls and in those from thyroidectomized animals treated with 1.8 μg of L-thyroxine, it had no effect on the adrenalin action in the tissues from thyroidectomized animals treated with either 0 or 0.1 μg of L-thyroxine. Contrary to what happened in the absence of glu-

Table 3
Effects of thyroidectomy and treatment with L-thyroxine on the formation of $^{14}CO_2$ by rat epididymal fat pads incubated in vitro in the presence of [1-^{14}C]glycerol. (See legend to table 2 for explanation.)

Group	Daily dose thyroxine (μg/100 g body wt.)	$^{14}CO_2$ (% of initial radioactivity/100 mg tissue)						
		Basal	Insulin	*P'*	Adrenalin	*P'*	Ins + Adr	*P'*
(a) no glucose in the medium								
Intact controls	0	5.39 ± 0.97	4.25 ± 0.67	N.S.	1.98 ± 0.48	<0.01	2.96 ± 0.46	<0.05
Thyroidectomized	0	9.60 ± 1.76	6.75 ± 0.78	N.S.	2.71 ± 0.49	<0.01	4.90 ± 1.23	<0.05
P		<0.05	<0.05		N.S.		N.S.	
Thyroidectomized	0.1	5.48 ± 1.12	6.01 ± 1.83	N.S.	3.44 ± 0.98	N.S.	5.90 ± 1.39	N.S.
P		N.S.	N.S.		N.S.		<0.05	
Thyroidectomized	1.8	3.75 ± 0.98	3.37 ± 0.69	N.S.	2.04 ± 0.44	N.S.	1.78 ± 0.33	N.S.
P		N.S.	N.S.		N.S.		N.S.	
(b) 5 mM glucose in the medium								
Intact controls	0	4.84 ± 0.70	6.60 ± 0.98	N.S.	1.27 ± 0.14	<0.001	1.60 ± 0.26 *	<0.001
Thyroidectomized	0	5.47 ± 0.75	10.7 ± 1.30 *	<0.01	2.92 ± 0.35	<0.01	6.24 ± 1.35	N.S.
P		N.S.	<0.05		<0.01		<0.001	
Thyroidectomized	0.1	6.44 ± 1.50	7.64 ± 0.94	N.S.	2.72 ± 0.41	<0.05	4.42 ± 0.67	N.S.
P		N.S.	N.S.		<0.01		<0.01	
Thyroidectomized	1.8	4.51 ± 0.79	7.88 ± 1.73 *	N.S.	1.10 ± 0.24	<0.001	1.51 ± 0.39	<0.01
P		N.S.	N.S.		N.S.		N.S.	

Table 4
Effect of thyroidectomy and treatment with L-thyroxine on the in vitro formation of ^{14}C-labelled fatty acids from [1-^{14}C]glycerol by rat epididymal fat-pads. (See legend to table 2 for explanation.)

Group	Daily dose thyroxine (μg/100 g body wt.)	Formation of ^{14}C-labelled fatty acids (%/100 mg)						
		Basal	Insulin	P'	Adrenalin	P'	Ins + Adr	P'
(a) no glucose in the medium								
Intact controls	0	0.42 ± 0.11	0.45 ± 0.17	N.S.	0.15 ± 0.06	<0.05	0.21 ± 0.07	N.S.
Thyroidectomized	0	0.41 ± 0.13	0.44 ± 0.16	N.S.	0.16 ± 0.04	N.S.	0.66 ± 0.36	N.S.
P		N.S.	N.S.		N.S.		N.S.	
Thyroidectomized	0.1	0.61 ± 0.20	0.71 ± 0.26	N.S.	0.24 ± 0.08	N.S.	0.46 ± 0.17	N.S.
P		N.S.	N.S.		N.S.		N.S.	
Thyroidectomized	1.8	0.12 ± 0.03	0.11 ± 0.04	N.S.	0.09 ± 0.07	N.S.	0.07 ± 0.03	N.S.
P		N.S.	N.S.		N.S.		N.S.	
(b) 5 mM glucose in the medium								
Intact controls	0	2.10 ± 0.43 ***	6.39 ± 0.90 ***	<0.001	0.25 ± 0.04	<0.001	0.95 ± 0.21 **	<0.05
Thyroidectomized	0	3.01 ± 0.68 ***	6.37 ± 1.73 **	N.S.	0.61 ± 0.19 *	<0.01	3.43 ± 0.95 **	N.S.
P		N.S.	N.S.		<0.05		<0.01	
Thyroidectomized	0.1	2.22 ± 0.66 *	7.39 ± 1.35 ***	<0.01	0.58 ± 0.18	<0.05	4.62 ± 1.10 **	N.S.
P		N.S.	N.S.		<0.05		<0.001	
Thyroidectomized	1.8	1.47 ± 0.35 **	4.97 ± 0.98 ***	<0.01	0.19 ± 0.12	<0.01	0.79 ± 0.27 *	N.S.
P		N.S.	N.S.		N.S.		N.S.	

Table 5
Effect of thyroidectomy and treatment with L-thyroxine on the in vitro synthesis of ^{14}C-labelled glyceride glycerol from [1-^{14}C]glycerol by rat epididymal fat pads. (See legend to table 2 for explanation)

Group	Daily dose thyroxine (μg/100 g body wt.)	Formation of ^{14}C-labelled glyceride glycerol (%/100 mg)							
		Basal	Insulin	*P'*	Adrenalin	*P'*	Ins + Adr	*P'*
(a) no glucose in the medium									
Intact controls	0	15.7 ± 2.7	17.2 ± 2.9	N.S.	4.25 ± 1.10	<0.001	7.00 ± 0.92	<0.01
Thyroidectomized	0	34.6 ± 3.8	33.6 ± 4.4	N.S.	25.1 ± 3.9	N.S.	28.3 ± 5.0	<0.05
P		<0.001	<0.01		<0.001		<0.001	
Thyroidectomized	0.1	25.0 ± 3.6	25.0 ± 6.1	N.S.	15.4 ± 3.2	N.S.	23.3 ± 4.7	N.S.
P		N.S.	N.S.		<0.001		<0.001	
Thyroidectomized	1.8	13.4 ± 4.7	10.7 ± 1.1		3.56 ± 1.2	<0.001	7.39 ± 2.74	N.S.
P		N.S.	N.S.		N.S.		N.S.	
(b) 5 mM glucose in the medium									
Intact controls	0	7.21 ± 1.32 **	3.58 ± 0.99 ***	<0.05	2.21 ± 0.38	<0.001	1.68 ± 0.21 ***	<0.001
Thyroidectomized	0	11.9 ± 2.0 ***	4.43 ± 0.82 ***	<0.01	10.0 ± 1.8 **	N.S.	3.96 ± 0.64 ***	<0.001
P		N.S.	N.S.		<0.001		<0.01	
Thyroidectomized	0.1	9.71 ± 2.62 **	3.94 ± 0.68 **	<0.05	7.15 ± 1.78 *	N.S.	3.09 ± 0.59 ***	<0.05
P		N.S.	N.S.		<0.01		<0.01	
Thyroidectomized	1.8	6.43 ± 1.35	3.09 ± 0.71 ***	<0.05	2.19 ± 0.48	<0.01	1.41 ± 0.34 *	<0.01
P		N.S.	N.S.		N.S.		N.S.	

cose in the medium, in the presence of glucose there were no significant differences among the groups with regard to the formation of glyceride glycerol, either in basal conditions or in the presence of insulin. Adrenalin, on the other hand, produced a significant reduction in the formation of glyceride glycerol in the tissues from the intact controls and in those from the thyroidectomized rats treated with 1.8 μg of L-thyroxine but it did not affect this parameter in the tissues from thyroidectomized rats treated with either 0 or 0.1 μg of L-thyroxine (table 5). The inhibitory effects of adrenalin and insulin on the formation of glyceride glycerol in the tissues from the controls incubated in the presence of glucose were augmented when both hormones were present in the incubation medium. In the thyroidectomized animals treated with either 0 or 0.1 μg of L-thyroxine, however, the formation of glyceride glycerol from glycerol in the presence of glucose, adrenalin and insulin together, appeared to be very similar to that observed with only glucose and insulin (table 5).

DISCUSSION

Glycerol metabolism was studied in adipose tissue of hypothyroid rats. Severe hypothyroidism was attained by thyroidectomy and low iodine diet, as shown by the low plasma levels of protein-bound iodine and growth retardation. The daily administration of very small doses of L-thyroxine (0.1 μg/100 g body wt./day) maintained the hypothyroid state of the animals as reflected by the low levels of plasma protein-bound iodine, but growth retardation was milder. The epididymal fat pads from these hypothyroid rats were small and had high DNA-P concentrations when compared with those of the intact control rats, which indicates that the size of adipocytes in hypothyroid animals is smaller. These differences disappear when the adipocyte size is corrected for differences in body weight. These considerations make it difficult, however, to determine the proper expression for intergroup comparisons. It was decided to express the results per unit of fresh tissue wt., because with this standardization, tissues from the different groups of animals studied released the same amount of glycerol during the 120 min incubation. This avoids also differences in the isotopic dilution of the [1-^{14}C]glycerol in the media among the groups.

Glycerol metabolism in adipose tissue from the intact hypothyroid controls incubated either under basal conditions or in the presence of glucose, insulin or adrenalin was very similar to that previously observed in normal rats (Dominguez and Herrera, 1976b). The results observed in the thyroidectomized rats treated with 1.8 μg L-thyroxine did not differ from those of their controls, suggesting that the changes observed in the hypothyroid animals are independent of the thyroidectomy itself.

The effect of adrenalin in increasing glycerol release by the tissues from the hypothyroid animals was similar to that in the controls. This agrees with the data of others (Rosenfeld and Rosenberg, 1965; Goodman and Bray, 1966), and indicates

that the lipolytic response of tissues from hypothyroid animals is comparable to that of tissue from normal animals if sufficient adrenalin is present.

Considering the amount of [1-^{14}C]glycerol converted to CO_2 and lipids (fatty acids and glyceride glycerol), the metabolism of glycerol by adipose tissue from fed hypothyroid rats was much greater than that from their controls. This agrees with previous results in fasted animals (Montoya and Herrera, 1974). The most significant effect observed in the tissues from hypothyroid rats was the enhanced synthesis of glyceride glycerol from glycerol, which explains the increased re-esterification of free fatty acids found by Fisher and Ball (1967) in similar preparations incubated in the absence of glucose. Very probably, the content of glycogen in the adipose tissue from these animals is insufficient as an endogenous source for α-glycerophosphate and the elevated esterification of fatty acids is caused mainly by the augmented utilization of glycerol. The formation of glyceride glycerol from glucose is increased in the adipose tissue from thyroidectomized rats (Bray and Goodman, 1968), which might explain the smaller effect of thyroidectomy on the synthesis of glyceride glycerol from glycerol when the tissue was incubated in the presence of glucose. This agrees well with the observation that, in the presence of insulin, the inhibiting effect of glucose on the formation of glyceride glycerol from glycerol is enhanced in all groups, because in this condition the uptake and metabolization of glucose by the tissues appears to be augmented (Cahill et al., 1960; Flatt and Ball, 1964; Bray, 1967).

Adrenalin is known to increase the rate of glyceride glycerol synthesis from glucose (Dominguez and Herrera, 1975) and this is not affected by thyroidectomy (Bray and Goodman, 1968). However, we found in the present experiments that although adrenalin reduces the formation of glyceride glycerol from glycerol, this effect was not observed in the hypothyroid animals. This observation in the presence of the normal sensitivity of glycerol release in the same tissues, demonstrates the dissociation between the changes in lipolysis and glycerol utilization in the adipose tissue. A similar dissociation was previously observed for the production of free fatty acids and the utilization of glucose in the same tissue (Bray and Goodman, 1968; Dominguez and Herrera, 1975).

The synthesis of fatty acids from glycerol is only meaningful in the presence of glucose in the medium, which provides the coenzymes for lipogenesis. In this condition, the antilipogenetic effect of adrenalin was smaller in the tissues from the hypothyroid rats than in those from their controls, either in the absence or in the presence of insulin. This, together with the results discussed above, suggests that, with the doses used in the present study, adipose tissue from thyroidectomized rats responds normally to the catabolic effects of adrenalin on glycerol metabolism (i.e., glycerol production) but the response to its inhibition of the anabolic pathways (i.e., glycerol utilization) is diminished. These changes in sensitivity to adrenalin in the presence of the augmented capacity of adipose tissue from hypothyroid animals to metabolize glycerol, together with the increased glycerogenesis suggested by Zederman et al. (1972), and the increased ability to synthesize glyceride glycerol

from glucose (Bray and Goodman, 1968) could contribute very actively to the favored fat retention demonstrated in these animals (Scow, 1951), while at the same time allowing them to survive starvation at the expense of a greater-than-normal fat mobilization (Aranda et al., 1972).

Doses of 0.1 μg of thyroxine/100 g body wt./day were sufficient to normalize some, but not all, of the parameters studied in the thyroidectomized animals, which emphasizes our previous suggestion (Aranda et al., 1972) that there is a wide variation in sensitivity to thyroid hormones of several biological responses, adipose tissue metabolism included.

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