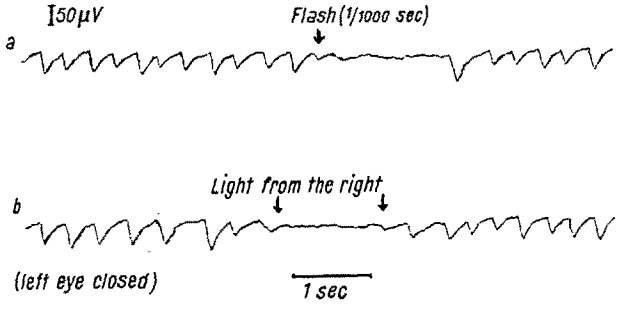
nystagmus for approximately $1^1/_2$ s. The same effect was produced on experiments with diffuse glare. Optokinetic nystagmus then ceased for as long as the eye was affected by the exposure (see Fig. *a* and *b*).



Effect of glare on optokinetic nystagmus

Comments.—The fact that optokinetic nystagmus abolished tends to show that the retinal sensitivity was reduced to such an extend that the moving stripes on the optokinetic screen could no longer be seen. The eye movement evoked by the optic stimulus therefore ceased. The method introduced in the above may be characterised as highly objective and rather appropriate for more exhaustive studies of the glare effect (for example under the influence of various pharmaceutics).

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Zusammenfassung

Mit Hilfe des optokinetischen Nystagmus und Elektronystagmographie kann man den Blendungseffekt auf das Sehvermögen bestimmen. Während der Blendung hören die optokinetischen Augenbewegungen auf. Der Effekt eines photographischen Blitzes (1/1000 s) und von schräg in das Auge fallendem Licht wurden studiert.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Effect of Single and Chronic Thyroxine Injection on Fatty Acid and Cholesterol Synthesis in Mice[1]

By Paola Marchi[2] and J. Mayer[3]

The action of thyroid hormone treatment on fatty acid synthesis from acetate has been previously investigated by Spirtes, Medes, and Weinhouse[4] in liver slices of rats made hyperthyroid. These authors found that the rate of oxidation of acetate was 30–70% higher in liver slices of thyroid hormone-treated rats. They also reported that contrary to expectation acetate incorporation into fatty acids in these treated rats was as high as in normal animals or higher.

Investigation on the synthesis of cholesterol in hyperthyroidism has been somewhat more extensive. Several authors have concluded that cholesterol synthesis is stimulated in hyperthyroid states while it appears depressed in the opposite condition[5–7].

It has been customary to study the effect of thyroid hormones in synthetic processes by including thyroid preparations in the diet. However, single determinations after chronic treatment are difficult to interpret as secondary physiological reactions may interfere with the initial effect of thyroxine. It seemed useful to determine the course of fatty acid and cholesterol synthesis from acetate after subcutaneous administration of a single dose of Thyroxine. A study of the effect of prolonged treatment is also included.

Materials and Methods. The animals used were adult male albino mice. They were maintained in individual cages and fed *ad libitum* Purina chow and water. All animals were kept fasting for 24 h before being killed.

Each group consisted of experimental animals and controls. Experimental animals received a subcutaneous injection of 100 μg of l-Thyroxine. Controls received an equal volume of saline by the same route. At the appropriate time all animals were injected intraperitoneally with 0·4 mg of Na acetate-C^{14} (an approximate total of 10^6 counts/min to each animal). 30 min later they were killed by a blow on the head and decapitation. The liver was excised and weighed separately from the carcass.

One group of animals received 1·8 mg of l-Thyroxine at 24 h intervals over a period of 28 days. An equal number of controls were injected with saline in the same way.

Thyroxine solution was prepared by dispersing the powder (l-sodium thyroxine pentahydrate, Smith, Kline, and French) in small amounts of 0·9% saline and adding 1 *N* sodium hydroxide until complete solubility was achieved. The pH was then adjusted to 8 with 1 *N* HCl and 0·9% saline added to obtain the desired volume. All injections were administered subcutaneously in volume of 0·2 ml.

Extraction and Determination of Fatty Acids and Cholesterol. The method followed was essentially that described in a previous publication[8]. Results are expressed as percentage of counts retained $\times 10^3$.

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Table I. Effect of Thyroxine on Hepatic Lipogenesis and Cholesterogenesis from Labelled Acetate

Interval Tx-sacrification	Groups (Number of animals)	Liver				
		Weight (g)	g Fatty acids per 100 g liver	% retention in liver Fatty acids × 1000	mg Cholesterol per g of liver	% retention in liver Cholesterol
6 h	Controls (7)	0·98 ± 0·06	9·41 ± 2·1	13·4 ± 3·6		
	Treated (7)	1·02 ± 0·12	8·94 ± 2·9	17·7 ± 6·8		
16 h	Controls (10)	1·19 ± 0·14	5·22 ± 1·7	20·2 ± 5·0	1·87 ± 0·51	9·1 ± 7·6
	Treated (10)	1·38 ± 0·25	5·25 ± 2·4	31·4 ± 19*	2·37 ± 0·35	20·8 ± 19
24 h	Controls (10)	1·55 ± 0·15	6·79 ± 1·9	24·7 ± 3·8	2·67 ± 0·41	32·0 ± 18
	Treated (10)	1·66 ± 0·18	8·80 ± 4·8	41·3 ± 8·6++	2·09 ± 0·20	161 ± 155**
32 h	Controls (10)	1·42 ± 0·23	6·83 ± 2·5	19·1 ± 8·0	2·57 ± 0·32	24·2 ± 16
	Treated (10)	1·48 ± 0·30	10·64 ± 4·2	23·6 ± 12	2·18 ± 0·56	104 ± 85+
48 h	Controls (10)	1·68 ± 0·47	8·69 ± 4·3	20·6 ± 6·6	2·45 ± 0·53	36·1 ± 19
	Treated (10)	1·64 ± 0·33	9·40 ± 5·5	22·0 ± 14	1·91 ± 0·51	141 ± 122***
Chronic Expt.	Controls (10)	1·48 ± 0·31	4·35 ± 2·0	13·3 ± 3·9	2·57 ± 0·40	31·0 ± 16
	Treated (9)	2·02 ± 0·40+	3·43 ± 1·9	59·9 ± 32++	1·88 ± 0·49	39·1 ± 51

* $p < 0{\cdot}10$ ** $p < 0{\cdot}05$ *** $p < 0{\cdot}02$ + $p < 0{\cdot}01$ ++ $p < 0{\cdot}001$

Results expressed as means S.D. in the livers of 6 groups of fasting mice, each consisting of treated animals and controls. The treated mice of the first 5 groups received 100 mg of Thyroxine subcutaneously and were sacrified with the controls (injected with saline in the same manner) at various intervals 30 min after an intraperitoneal injection of acetate-C^{14}. The treated mice of the last group received 1·8 mg of l-Thyroxine over a period of 28 days.

Table II. Effect of Thyroxine on Carcass Lipogenesis and Cholesterogenesis from Labelled Acetate

Interval Tx-sacrification	Groups (Number of animals)	Carcass				
		Weight (g)	mg Fatty Acids per 100 g carcass	% retention in carcass Fatty acids × 1000	mg Cholesterol per 100 g carcass	% retention in carcass Cholesterol × 1000
6 h	Controls (7)	21·1 ± 1·5	10·8 ± 3·9	260 ± 66		
	Treated (7)	21·1 ± 1·4	10·8 ± 3·7	362 ± 84**		
16 h	Controls (10)	21·9 ± 1·9	7·5 ± 3·9	268 ± 32	241	290 ± 48
	Treated (10)	21·3 ± 2·1	6·1 ± 1·7	341 ± 57++	230	328 ± 56
24 h	Controls (10)	24·1 ± 1·9	6·5 ± 1·7	296 ± 53	226	321 ± 72
	Treated (9)	24·8 ± 1·6	7·1 ± 2·2	287 ± 52	244	403 ± 90**
36 h	Controls (10)	24·7 ± 2·3	7·9 ± 3·0	257 ± 62	257	298 ± 58
	Treated (9)	24·1 ± 2·0	7·8 ± 3·0	262 ± 50	256	267 ± 28
48 h	Controls (10)	25·4 ± 1·7	8·2 ± 3·9	227 ± 40	244	301 ± 67
	Treated (10)	25·5 ± 2·2	9·5 ± 5·4	221 ± 19	238	298 ± 41
Chronic Expt.	Controls (10)	25·6 ± 2·6	5·3 ± 2·1	236 ± 32	278	378 ± 96
	Treated (9)	28·2 ± 2·2	4·0 ± 2·3	209 ± 34	266	280 ± 30+

* $p < 0{\cdot}10$ ** $p < 0{\cdot}05$ *** $p < 0{\cdot}02$ + $p < 0{\cdot}01$ ++ $p < 0{\cdot}001$

Results expressed as means S.D. found in the carcasses of the same animals as in Table I

Results. Hepatic incorporation of acetate-C^{14} into fatty acids and cholesterol after one subcutaneous dose of 100 g of l-Thyroxine. As can be seen from Table I hepatic lipogenesis appears susceptible of being stimulated by one dose of Thyroxine. 24 h after administration of a single dose the rise in lipogenesis was 70% and highly significant. Within 48 h the effect had practically disappeared as can be readily observed from Table I. The % retention of acetate-C^{14} into liver cholesterol appears to be also increased by one subcutaneous injection of Thyroxine. A 3–5-fold increase was observed between 24 and 48 h after the hormone administration. Contrary to what was observed for fatty acid synthesis, the effect of thyroxine on liver cholesterogenesis appears to be more persistent: the increase in rate of cholesterogenesis was still several times the normal rate after 48 h. In spite of the fact that the significance of the increase is partly obscured by the wide range of values, it seems possible to conclude that at 24, 36, and 48 h after Thyroxine administration liver cholesterogenesis is decidedly higher than in the control group.

Carcass incorporation of acetate-C^{14} into fatty acids and cholesterol after one subcutaneous dose of 100 g of l-Thyroxine. Table II shows carcass retention of acetate-C^{14} into fatty acids to be affected in the way of an increase 6 and 16 h after Thyroxine injection, while the incorporation into carcass cholesterol, except for a small and transient rise found 24 h after the administration of the hormone was not affected in the treated groups at other time intervals.

Hepatic incorporation of acetate-C^{14} into fatty acids and cholesterol after repeated administration of l-Thyroxine. The animals which received 1·8 mg of l-Thyroxine over a period of 28 days did not show some of the 'classic' signs of hyperthyroidism. Their weight was increased significantly (p 0·01) over that of the controls and their fur appeared more luxuriant and cleaner than that of the controls. Food intake was increased. While the increase in body weight was in part due to the increase in liver weight, the weight of the carcass was also greater at the end of treatment. Hepatic lipogenesis was increased four-fold. The increase was highly significant as is readily seen from Table I. While, as mentioned above, liver weight was increased, the fatty acid content per 100 g of liver was significantly lower indicating a faster turnover of hepatic fatty acids. Table I also shows that contrary to what could be expected, cholesterol synthesis *in vivo* following chronic administration of Thyroxine was not increased.

Carcass incorporation of acetate-C^{14} into fatty acids and cholesterol after repeated administration of l-Thyroxine. Table II shows that following prolonged thyroxine administration carcass lipogenesis was not affected, while cholesterol synthesis tended to be depressed.

Discussion. In all experiments the animals were kept fasted for 24 h. The duration of fasting had been chosen so as to minimize the extremely wide range of individual variability seen when lipogenesis and cholesterogenesis are measured in the fed state. Under these conditions, administration of a single dose of thyroxine appeared to promote within 24 h a 79% increase in hepatic synthesis of fatty acids and a 3–5-fold increase in the hepatic synthesis of cholesterol.

A major limitation in interpreting these experiments is that data on retention of acetate-C^{14} into fatty acids and cholesterol does not provide a satisfactory measure of lipogenesis and cholesterologenesis unless the specific activity of the pool of acetyl groups is known at the site of synthesis. It is possible that the apparent rates of synthesis are in part function of the variations in the dilution of the administered acetate-C^{14} by endogenously produced acetate. Such a possibility has been examined experimentally in studies of lipogenesis and cholesterogenesis in the hereditary obese hyperglycemic syndrome and in hypothalamic obesity in mice[9]. In a situation such as the one created by thyroid treatment where increased oxygen consumption represents the most evident and rapid feature even after the administration of one single dose, a reduced acetyl pool is hardly to be expected. It is likely that the results would be even more significant because of increased size of acetate pool in the treated groups if the size of these pools had in fact been determined.

It seems therefore reasonable to conclude that one effect of thyroxine is that it increases the rate of fatty acid synthesis in the mouse liver. This in turn may be coupled with or rather be the result of an increased glucose oxidation as both liver glucose uptake by isolated rat liver[10] and glucose oxidation in rat liver slices[11] have been found to be increased by a period of thyroid treatment. GLOCK *et al.*[11,12] have also found significantly higher levels of activity of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the livers of thyroid treated rats. This would support the possibility that the increased lipogenesis might find its explanation in an increased HMP shunt activity. However, further investigations where utilization of 1-^{14}C-glucose and 6-^{14}C-glucose liver slices were determined by measuring the conversion of these labelled substrates into $^{14}CO_2$ failed to show comparatively greater participation of the HMP shunt in the liver slices of the thyroid treated rats. Total liver TPN and principally TPNH were strikingly reduced as a result of thyroxine treatment. Thus, while the increase in lipogenesis in the thyroxine-treated animals is in agreement with the general concept that lipogenesis is coupled with an increased glucose utilization, the mechanism of the stimulation remains obscure. Explanation of the fact that after initially stimulating cholesterogenesis, continued thyroxine treatment brings cholesterogenesis back to pre-treatment levels also requires further experimental work.

Résumé

L'effet d'une dose souscutanée de l-Thyroxine sur des souris à jeun consiste en une augmentation progressive de la lipogénèse et de la cholestérogénèse hépatiques. Cette augmentation atteint 70% pour la lipogénèse après 24 h et 300–500% pour la cholesterogénèse après 24, 36 et 48 h.

Un traitement chronique (28 j) cause une augmentation (400%) de la lipogénèse, mais non de la cholestérogénèse hépatiques.

Les effets de la thyroxine sur la lipogénèse et la cholestérogénèse extra-hépatiques sont beaucoup moins nets.

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