The Effect of Thyroidectomy on the Pattern of Fatty Acids Synthesized by Mammary Gland from Lactating Rats

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(Received 22 September 1966)

Thyroidectomy decreased the content of short-chain fatty acids and increased the content of long-chain fatty acids in the mammary glands of lactating rats. This effect was replicated in the glands of untreated rats limited to the same food intake as the thyroidectomized animals. Thyroidectomy decreased the incorporation of [6-¹⁴C]glucose into short-chain fatty acids and increased the incorporation into longer-chain acids. Restriction of the food intake of untreated animals did not cause a similar shift of the incorporation pattern. The possibility that the thyroxine effect on lipogenesis is secondary to its effect on carbohydrate metabolism is discussed.

Thyroid hormone is known to play a role of some importance in lipogenesis. Thyroidectomy decreases the rate of hepatic fatty acid synthesis (Bates, Zomzely & Mayer, 1955; Fain & Wilhelmi, 1962; see Masoro, 1962), whereas the injection of physiological doses of thyroxine stimulates lipogenesis (Spirtes, Medes & Weinhouse, 1953; Fletcher & Myant, 1958; Marchi & Mayer, 1959) and alters the pattern of the fatty acids formed in the livers of rats so treated in favour of a higher content of long-chain fatty acids and oleic acid (Ellefson & Mason, 1964). It also increases the rates of synthesis of these long-chain acids (Gompertz & Greenbaum, 1966). Thyroxine also promotes the initiation and maintenance of milk secretion in rats (Desclin, 1949) and ruminants (Folley & Young, 1938, 1939; see Cowie, 1961), whereas thyroidectomy decreases mammary-gland function (see Cowie, 1961). Further, the activity of the pentose phosphate pathway, which is intimately linked with the rate of lipogenesis in mammary tissue (Glock & McLean, 1953, 1958; Abraham, Hirsch & Chaikoff, 1954; McLean, 1958; Abraham & Chaikoff, 1959), is diminished in this tissue after thyroidectomy (E. Walters & P. McLean, unpublished work).

Since thyroxine appears to be intimately concerned in both the maintenance of lactation and the control of lipogenesis in other tissues it was decided to investigate the effect of thyroidectomy on the pattern of fatty acid synthesis in the mammary glands of lactating rats.

METHODS

Animals. Primiparous albino rats of body weight 150–250 g. were thyroidectomized, or sham-operated, on the fourth day of lactation under ether anaesthesia. Litters were restricted to eight pups. The animals were allocated into one of three groups: (1) sham-operated controls, allowed food *ad lib.*; (2) sham-operated controls, pair-fed with the thyroidectomized rats; (3) thyroidectomized rats, allowed food *ad lib.* Thyroidectomized lactating animals eat considerably less than the sham-operated controls and this necessitates the control pair-fed group, since it is known that restriction of the food intake leads to impaired milk secretion (Smith & Dastur, 1938). Since thyroidectomy perforce leads also to parathyroidectomy, as the parathyroids are embedded in the thyroid, all thyroidectomized animals received calcium gluconate in their drinking water. Blood calcium measurements showed that this treatment restored the blood calcium concentration to normal (controls, 4.10 mg./100 ml.; thyroidectomized, 4.07 mg./100 ml.).

The rats were killed on the eleventh or twelfth day of lactation and the abdominal mammary glands removed.

Incubation of mammary-gland slices. Mammary-gland slices (500 mg.) were incubated in 4.5 ml. of Krebs–Ringer bicarbonate (Umbreit, Burris & Stauffer, 1957), containing 100 μ moles of unlabelled glucose and 2.0 μ C of [6-¹⁴C]glucose, for 1 hr. at 37°. The gas phase was O₂ + CO₂ (95:5). At the end of this time the reaction was stopped by the addition of 1 ml. of 5N-HCl.

Extraction of fatty acids. The slices and the medium were transferred to a stoppered tube, 10 ml. of ethanolic 10% (w/v) KOH was added and the whole was heated at 75° for 5 hr. At the end of this time 10 ml. of water was added and the non-saponifiable fraction extracted with 3 × 15 ml.

of light petroleum (b.p. 40–60°). This fraction was discarded. The residual aqueous layer was then acidified with H_2SO_4 and re-extracted with 3×15 ml. of light petroleum. The combined petroleum extract was washed with 2×25 ml. of 0.05N- H_2SO_4 and one-tenth of the volume was taken for counting in a Nuclear-Chicago Corp. scintillation counter (series 701), the counts being corrected to disintegrations/min. by the channels-ratio method. The remainder was dried over Na_2SO_4 and then concentrated in a rotary evaporator, under a stream of N_2 , for use in the chromatographic procedure.

Gas-liquid chromatography. A sample (usually about one-third) of the light-petroleum extract was dried in a stream of N_2 and 5–10 μl . of the residual oil was applied to a column [150 cm. $\times$ 0.4 cm. containing 10% (w/w) of diethylene glycol adipate on 100–120 mesh Celite that had been treated with 2% phosphoric acid]. The analyses were performed on a Pye Panchromatograph (W. G. Pye and Co. Ltd., Cambridge) fitted with an argon-ionization mass detector, by using a stream splitter (300:1) and the continuous-flow counting system of James & Piper (1961). The analyses were temperature-programmed to rise from 120° to 195° by 2°/min.

The recorded chromatograms were interpreted as follows. On the mass tracing the area of each peak was calculated by triangulation. The radioactivity tracing was in the integral form and the radioactivity of each peak

could readily be ascertained by means of a pair of parallel rulers.

RESULTS AND DISCUSSION

After mammary-gland slices had been incubated in the presence of $[6\text{-}^{14}\text{C}]\text{glucose}$ radioactivity was only occasionally detected in fatty acids of less than 8 carbon atoms in length and the amounts were small. At the other end of the scale, no radioactivity was detected in any of the acids of chain length greater than 16 carbon atoms. This latter observation is in accord with the work of Popják, French, Hunter & Martin (1951), who failed to find appreciable incorporation of radioactivity into the C_{18} fatty acids of milk fat when labelled acetate was used as substrate.

The distribution of the radioactivity in the fatty acids in mammary tissue is shown in Table 1.

Thyroidectomy appears to cause a shift in the pattern of fatty acids present in favour of the longer-chain fatty acids. The amounts of C_8 , C_{10} and C_{12} acids present in the mammary tissue of thyroidectomized rats is less than in the free-fed control animals, whereas the amount of C_{16} acid

Table 1. *Relative proportions and rates of formation of the fatty acids in mammary tissue from normal and thyroidectomized lactating rats*

The relative proportion of each fatty acid and the ^{14}C incorporation are each expressed as the percentage of that fatty acid of the total $\text{C}_8 + \text{C}_{10} + \text{C}_{12} + \text{C}_{14} + \text{C}_{16}$ fatty acids. The total counts incorporated/500 mg. of mammary slices/hr. were: (a) free-fed controls, 1.049×10^6 disintegrations/min.; (b) pair-fed controls, 0.883×10^6 disintegrations/min.; (c) thyroidectomized, 0.604×10^6 disintegrations/min.; the relative proportions given are calculated as percentages as given above. N.S., Not significant.

	C_8	C_{10}	C_{12}	C_{14}	C_{16}
A. Relative proportions					
Controls (12) (fed <i>ad lib.</i>)	6.8 ± 0.56	17.6 ± 2.0	18.9 ± 2.7	16.5 ± 1.4	40.7 ± 4.0
Controls (6) (pair-fed)	4.8 ± 1.0	15.5 ± 1.0	13.3 ± 1.0	15.2 ± 1.6	51.9 ± 1.7
Thyroidectomized (11)	4.1 ± 0.5	14.8 ± 2.1	14.2 ± 0.9	15.7 ± 0.6	51.7 ± 3.7
P values:					
Controls versus pair-fed controls	N.S.	N.S.	N.S.	N.S.	0.058
Controls versus thyroidectomized	0.011	N.S.	N.S.	N.S.	0.061
Thyroidectomized versus pair-fed controls	N.S.	N.S.	N.S.	N.S.	N.S.
B. Relative incorporation of ^{14}C from $[6\text{-}^{14}\text{C}]\text{glucose}$					
Controls (12) (fed <i>ad lib.</i>)	13.3 ± 3.5	24.7 ± 3.8	26.2 ± 2.2	21.4 ± 1.8	14.3 ± 1.5
Controls (7) (pair-fed)	11.6 ± 2.0	27.2 ± 2.6	26.0 ± 1.5	19.7 ± 1.7	15.4 ± 0.7
Thyroidectomized (11)	5.5 ± 1.0	22.0 ± 3.3	23.2 ± 0.1	23.5 ± 0.8	26.2 ± 3.0
P values:					
Controls versus pair-fed controls	N.S.	N.S.	N.S.	N.S.	N.S.
Controls versus thyroidectomized	0.043	N.S.	N.S.	N.S.	< 0.001
Thyroidectomized versus pair-fed controls	0.018	N.S.	N.S.	0.036	0.009

is increased. Examination of the results for the pair-fed control animals, however, reveals that this change is almost certainly due to an inadequacy of the food intake rather than to a direct hormonal effect. That this is so is shown by the extraordinarily close correspondence of the values found for the pair-fed controls and thyroidectomized animals, both of which stand in sharp contrast with the free-fed controls. This finding also parallels the observation by Smith & Dastur (1938) that inanition causes a decrease in the molar proportion of short-chain acids. The values in Table 1 are given as percentages and do not reveal changes in absolute amounts that are not easily measured by the methods employed here.

Thyroidectomy appears to cause a shift of fatty acid synthesis in favour of the longer-chain fatty acids. The incorporation into C_8 acids is decreased by 60% and that into C_{16} acids is increased by 80%. However, in contrast with the effect of food restriction on composition, pair-feeding does not affect the incorporation pattern. The results for fatty acids from pair-fed control rats clearly resemble those for the free-fed controls; indeed, no statistical changes are evident and both differ from the thyroidectomized animals. However, although expression of the results on a percentage basis has revealed the change in pattern, it obscures the more important change. Calculation of the total incorporation of radioactivity into the palmitic acid peak shows that the counts incorporated into palmitate for the three groups were (thousands of disintegrations/min.): thyroidectomized, 158 ± 9 ; free-fed control, 150 ± 6 ; pair-fed control, 136 ± 8 ; i.e. the rate of synthesis of palmitate is essentially the same in all three groups, and the large rise in the percentage of counts in palmitate should more properly be ascribed to an inhibition of the rate of synthesis of the short-chain, more particularly of C_8 , fatty acids.

An inhibition of this kind in thyroidectomized rats might arise from a number of causes, but two of the more likely are (1) a decreased rate of generation of NADH during glycolysis, suppressing the formation of short-chain fatty acids, and (2) an increased cytoplasmic citrate concentration changing the fatty acid pattern.

Hele, Popják & Laurysens (1957) suggested that the mammary gland contained two systems for synthesizing fatty acids: an NADH-linked system synthesizing fatty acids up to C_8 and an NADPH-linked system synthesizing longer-chain acids. The existence of two systems has also been suggested by Singh & Kumar (1963) and Lachance & Morris (1965). Thus a decreased rate of glycolysis would preferentially inhibit the synthesis of short-chain fatty acids. There is evidence that glucose oxidation via the glycolytic pathway is decreased

in hypothyroid rats (see Hoch, 1962), and this may well account for the smaller incorporation in the shorter-chain acids.

A similar effect could be achieved by increased concentrations of cytoplasmic citrate. Smith & Dils (1966) have shown that citrate (6mM) added to a preparation consisting of the supernatant and microsomal fractions from rabbit mammary gland decreases the incorporation of acetate into C_4 , C_6 and C_8 fatty acids while increasing the incorporation into the C_{12} , C_{14} and C_{16} acids. Whether this is a specific effect of citrate or an effect of citrate inhibiting phosphofructokinase and hence operating by the above mechanism is not clear. The resemblance between the shift of fatty acid pattern found by Smith & Dils (1966) and that found in the present study suggests that a study of citrate metabolism in the hypothyroid animal might prove important in interpreting the facts reported here. It is, however, probable that an accumulation of citrate would not, in itself, completely explain the present findings, since Gompertz & Greenbaum (1966) observed that thyroxine still caused a shift of fatty acid pattern in liver, even in the presence of the higher concentration of citrate described by Smith & Dils (1966).

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