EFFECT OF PITUITARY HORMONES AND THYROXINE ON CITRATE-CLEAVAGE ENZYME IN LIVER AND ADIPOSE TISSUE

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Received November 17, 1965

There is considerable evidence that fatty acid synthesis is decreased in hypophysectomised animals and that lack of thyrotrophin, leading to a deficiency of thyroid hormone, is an important factor in this decreased fat synthesis (see Masoro, 1962). Evidence for this is provided by the observations that thyroxine will restore lipogenesis to normal in both hypophysectomised rats and thyroidectomised animals (Bates, Zomzely & Mayer, 1955; Dayton, Dayton, Drimmer & Kendall, 1960; Fain & Wilhelmi, 1962; Masoro, 1962). Further, adrenalectomy or ovariectomy results in little or no change in fatty acid synthesis in liver or adipose tissue, (Bates et al. 1955; Cohn & Joseph, 1957; Jeanrenaud & Renold, 1960).

The importance of citrate cleavage enzyme as a control point in fatty acid synthesis has recently been widely discussed (Srere, 1965; Kornacker & Lowenstein, 1965a). This enzyme has been shown to be depressed in liver in diabetes and to be restored to normal or higher than normal by insulin or fructose feeding (Kornacker & Lowenstein, 1965b), to alter in starvation and refeeding in a manner parallel to fat synthesis (Kornacker & Lowenstein, 1965a) and to be depressed in hypophysectomised rats (Abraham, Kopelovich & Chaikoff, 1964). It has been demonstrated that citrate cleavage enzyme in adipose tissue undergoes even more striking changes in diabetic rats than does the liver enzyme (Brown & McLean, 1965). This may be related to the

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more specialised function of adipose tissue in the formation of fatty acids. The general pattern that has emerged in both liver and adipose tissue is that changes in citrate-cleavage enzyme parallel alterations in fatty acid synthesis. For this reason citrate-cleavage enzyme has been studied in liver and adipose tissue of hypophysectomised and thyroidectomised rats and in these rats treated with physiological replacement doses of thyroxine (5-10µg./day). During the period of treatment the food intake was carefully controlled since there is evidence that the fall in fatty acid synthesis in hypophysectomised rats may be, in part, related to food intake and absorption (Goodman, 1964). The results of these experiments are shown in Fig. 1 and Table 1.

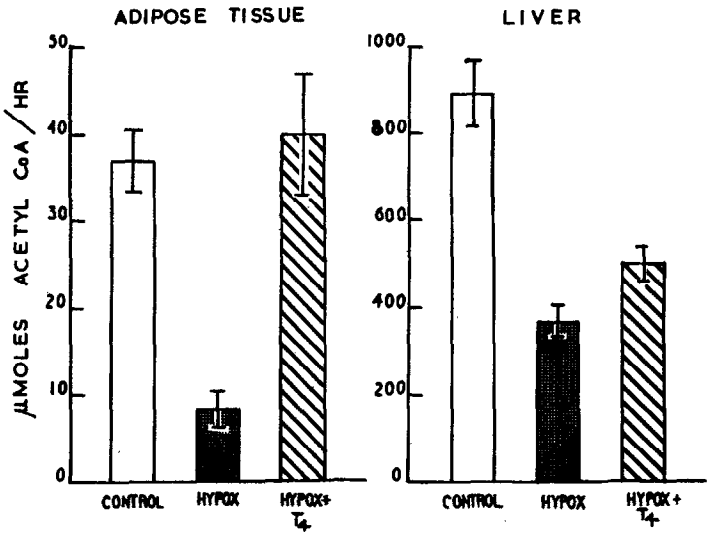

Fig. 1. The activity of citrate-cleavage enzyme in adipose tissue and liver of control, hypophysectomised and hypophysectomised thyroxine-treated rats.

Thyroxine treatment was commenced two weeks after hypophysectomy and 10 µg. L-thyroxine was given subcutaneously each day for 10 days. During this period of treatment the food intake of the three groups of animals was controlled so that the control and thyroxine-treated groups received the same amount of food as that eaten by the hypophysectomised rats. The activity of citrate-cleavage enzyme was measured by the acetohydroxamate method of Kornacker & Lowenstein (1965a) using high speed supernatant fractions (100,000 g. for 45 min.) prepared in a medium containing 150 mM KCl, 5 mM $MgCl_2$, 5mMEDTA, 10mM mercaptoethanol and adjusted to pH 7.4 with $KHCO_3$. The results are given as µmoles acetyl-CoA formed/hr. at 37° by one epididymal fat pad or by the entire liver. The mean values are shown by the columns, the vertical lines represented twice the SEM. Each group contained 8 animals.

TABLE I Effect of Thyroidectomy and Thyroxine Treatment on the Activity of Citrate-Cleavage Enzyme in Rat Liver

Experimental group	Body wt. (g.)	Citrate-Cleavage Enzyme (μmole Acetyl-CoA/hr.)	
		g. liver	Total Liver
Normal Control (8)	204±5	99±6	907±65
Thyroidectomized (9)	185±5	46±6 P<0.001	345±58 P<0.001
Thyroidectomized L-thyroxine 5 μg/day (7)	198±5	146±10 P 0.002	1270±115 P 0.018
Thyroidectomized L-thyroxine 25 μg/day (7)	179±3	151±2 P<0.001	1100±51 P 0.036

Thyroid deficiency was produced by thyroidectomy followed 10 days later by administration of I^{131}. Uptake in the neck region was used to eliminate rats with incomplete thyroid ablation. Two weeks after operation the rats were divided into the three groups shown. Treatment with thyroxine was continued for 10 days. During this latter period the food intake was controlled, the rats were limit fed, usually restricted to the food intake of the hypothyroid rats in each group of four animals. The methods used were as described in Fig. 1. Results are given as means ±SEM, figures in parentheses are the number of animals in each group. Fisher's P values are given for comparison of the experimental groups with the sham operated control group.

In hypophysectomised rats the activity of citrate-cleavage enzyme falls very markedly in adipose tissue to 23% of the control value and is completely restored by 10 days treatment with thyroxine (10μg./daily). These differences

are highly significant whether expressed on the basis of activity in the whole epididymal fat pad (as in Fig. 1) or as activity/mg. protein. The changes in the liver enzyme under these same conditions are somewhat different in that not only is the fall in citrate-cleavage enzyme in the hypophysectomised rats not so great but there is only partial restoration with thyroxine treatment. The activity of liver citrate-cleavage enzyme in the thyroidectomised rats was decreased to one half the normal value (Table 1) but in this case there was complete restoration with thyroxine treatment at both doses used (5 and 25μg. daily for 10 days). Murad & Freedland (1965) have shown that very large doses of thyroxine (1 mg./day for 5 days) will cause a substantial increase in the citrate-cleavage enzyme in normal rat liver.

Taken in conjunction these results suggest that thyroxine has a controlling influence on the activity of citrate-cleavage enzyme in both adipose tissue and liver. However, it would seem that another pituitary factor in addition to thyrotrophic hormone may be necessary for the full restoration of the liver citrate-cleavage enzyme in hypophysectomised rats. In this context it is of interest that Hill, Bauman & Chaikoff (1957) have shown that the conversion of hexose carbons to fatty acids by the liver requires the concurrence of more than one of the anterior pituitary hormones.

Kornacker & Lowenstein (1956b) have suggested that the availability of an intermediate in carbohydrate metabolism, such as α-glycerophosphate, may have a controlling influence on the activity of citrate-cleavage enzyme in liver. In the present studies it appeared that there might be a correlation between restoration of citrate-cleavage enzyme and the activity of glucose-ATP phosphotransferase in adipose tissue. It was found, in parallel studies, that the activity of this latter enzyme fell to 15% of the control value in the hypophysectomised group and that this was completely restored by thyroxine treatment. The values for the glucose-ATP phosphotransferase activity in adipose tissue of the control, hypophysectomised

and hypophysectomised-thyroxine treated groups were respectively 25±4; 3.6±0.6 and 17.4±1.2 μmoles glucose-6-phosphate produced/epididymal fat pad/hr. at 37°.

The changes in the activity of citrate-cleavage enzyme in different thyroid states correlates well with the known effect of physiological doses of thyroxine in increasing lipid synthesis in hypothyroid rats.

ACKNOWLEDGEMENTS

We are indebted to Mr. Keith Greenslade for skilled technical assistance. This work was in part supported by a grant to the Medical School from the British Empire Cancer Campaign.

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