RELATION OF CITRATE OXIDATION TO FATTY ACID SYNTHESIS IN LIVER AND LACTATING MAMMARY GLAND*

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It has been shown repeatedly that citrate and isocitrate stimulate fatty acid synthesis in liver homogenates (Brady and Gurin, 1952; Dituri et al., 1957; Porter et al., 1957). This effect cannot be ascribed solely to TPNH generation from these tricarboxylic acids (Abraham et al., 1959). In further studies we observed that glucose-6-phosphate greatly inhibited the conversion of the C^{14} of citrate-6-C^{14} to CO_2 by a particle-free supernatant fraction prepared from lactating rat mammary glands (Fig. 1). This observed inhibition could have been due to competition for TPN between isocitric dehydrogenase and glucose-6-phosphate oxidizing enzymes. An opposite phenomenon was reported earlier for rat liver homogenates--here citrate or isocitrate inhibited glucose-6-phosphate oxidation (Matthes et al., 1960). This difference between liver and mammary tissue can probably be explained on the basis of their enzyme contents. In the liver supernatant fraction, isocitric dehydrogenase is seven times more active than are the glucose-6-phosphate oxidizing enzymes (unpublished observations); in the same fraction prepared from lactating rat mammary glands, the latter enzymes were 12 times more active than was isocitric dehydrogenase.

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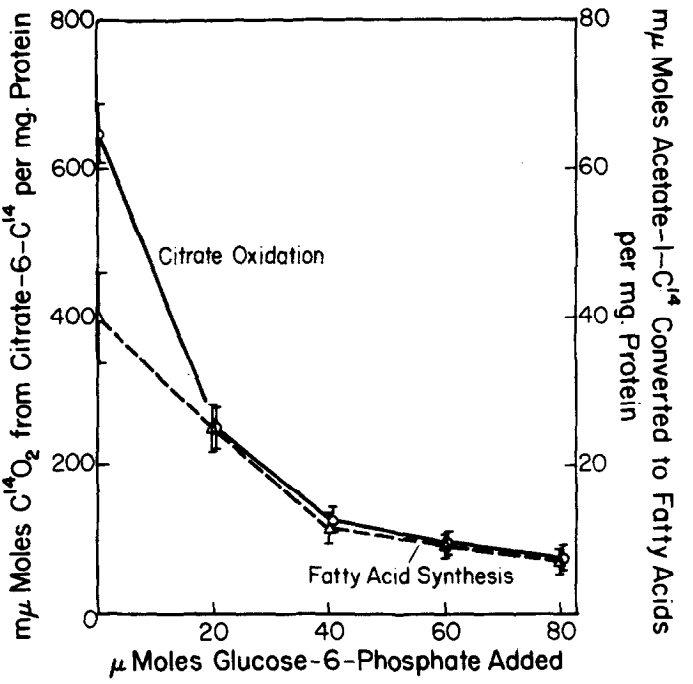

Fig. 1. Effect of Glucose-6-Phosphate on Conversion of C^{14} of Citrate-6-C^{14} to CO_2 and of Acetate-1-C^{14} to Fatty Acids.

1.5 ml. of a particle-free supernatant prepared from lactating rat mammary glands (10 g tissue in 30 ml of 0.25 M sucrose) were incubated for 2 hours at 30° with 240 μmoles glycylglycine buffer (pH 7.2), 10 μmoles $KHCO_3$, 70 μmoles $MgCl_2$, 60 μmoles reduced glutathione, 0.1 μmole CoA, 0.5 μmole TPN, 10 μmoles ATP, 50 μmoles potassium citrate, and 6 μmoles potassium acetate in a final volume of 3.5 ml. In the experiments with labeled citrate, each flask contained 9.7×10^3 CPM of C^{14}; in those with labeled acetate, 2.4×10^6. Each value is the average and standard error of results obtained with 12 rats.

Fatty acid synthesis by the particle-free supernatant fraction obtained from the lactating rat mammary gland homogenate showed a strict requirement for citrate and TPN (unpublished observations). We therefore studied the effect of inhibition of citrate oxidation by glucose-6-phosphate on fatty acid synthesis in this system. Even though the total amount of TPNH produced in the presence of glucose-6-phosphate was the same as or higher than that produced in its absence, the inhibition of citrate oxidation was accompanied by a decreased fatty acid synthesis from acetate (Fig. 1).

TABLE I

EFFECT OF TRANSACONITATE ON PRODUCTION OF TPNH FROM CITRATE BY RAT LIVER AND LACTATING RAT MAMMARY GLAND HOMOGENATE FRACTIONS

Assay mixture consisted of 2 ml of 0.15M glycylglycine buffer (pH 7.2 for mammary gland and pH 7.5 for liver), 60 μmoles citrate, 40 μmoles $MgCl_2$, 0.5 μmole TPN and 0.01 ml of particle-free supernatant fraction. 50 μmoles of transaconitate were added as indicated below.

Expt.	mumoles TPNH produced* per mg supernatant protein per min			
	Mammary gland		Liver	
	Citrate	Citrate plus transaconitate	Citrate	Citrate plus transaconitate
1	12.36	6.32	12.71	5.20
2	16.28	8.24	14.18	7.26

* The extinction coefficient used was 6.22×10^6 cm^2 per mole (Horecker and Kornberg, 1948).

TABLE II

EFFECT OF TRANSACONITATE ON CITRATE OXIDATION AND FATTY ACID SYNTHESIS BY RAT LIVER AND MAMMARY GLAND HOMOGENATE FRACTIONS

In addition to the incubation components described in the legend for Figure 1, the following were added in liver experiments: 1 μmole $MnCl_2$*, 38 μmoles ATP, 40 μmoles glucose-6-phosphate and 0.1 ml liver microsomes (8 mg protein). In the mammary gland experiments, 1 μmole of $MnCl_2$* was added. Each value is the average of results obtained with 3 rats.

Transaconitate added	mumoles labeled citrate converted to CO_2, and labeled acetate converted to fatty acids per mg supernatant protein			
	Liver		Mammary gland	
	CO_2	Fatty acids	CO_2	Fatty acids
μmoles				
0	860	8.6	1050	127
25	770	7.4	840	70
50	650	5.5	660	50
75	560	4.5	500	32

* The stimulation by manganese of fatty acid synthesis and citrate oxidation (compare results of Figure 1 with those in Table I) might be due to its effect on carboxylation of acetyl-CoA (Wakil, 1958) and decarboxylation of oxalosuccinate (Ochoa, 1955).

A similar effect on fatty acid synthesis from acetate by rat liver and mammary gland cell-free systems was observed when the conversion of citrate to isocitrate was inhibited by addition of transaconitate (Tables I and II).

These findings indicate that citrate oxidation affects lipogenesis by a mechanism other than, and in addition to that involving TPNH generation.

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