The Effect of Thyroxine Treatment on the Rate of Gluconeogenesis in the Perfused Rat Liver

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The rate of hepatic gluconeogenesis from many precursors is higher in starved (glycogen-depleted) than in well fed rats. Injection of thyroxine (1mg. daily for 5 days into rats weighing about 200g.) decreased the concentration of liver glycogen, from about 250μmoles to about 50μmoles/g. fresh wt. (expressed as glucose) when the animals had free access to food. After thyroxine treatment the following rates of glucose formation (μmoles/min./g.) were found:

Substrate added	Fed rats	Starved 48hr.
L-Lactate	1·0	1·6
Glycerol	1·1	1·5
Dihydroxyacetone	1·3	1·6
Fructose	1·6	—
Serine	0·4	—
Alanine	0·8	—

The comparison of these rates with those published previously (Ross, Hems & Krebs, 1967) for normal rats shows that thyroxine treatment raises the rate of gluconeogenesis from lactate and glycerol in the livers of fed or starved rats but has no effect with fructose and serine in well fed rats. Butyrate and glucagon singly accelerate the rate from lactate in treated fed and starved rats as well as in untreated starved animals. When present together their effects are additive in the starved untreated rats, greater than additive in the starved treated rats, and no greater than glucagon alone in the fed treated rats. The highest rates of glucose formation from lactate (3·2μmoles/min./g.) were found in starved thyroxine-treated animals after the addition of butyrate (2mM) and glucagon (1μg./ml.). Since the weight of the liver in relation to the total body wt. is about 15–20% greater in thyroxine-treated rats the effects of thyroxine treatment on gluconeogenesis are even greater when expressed per unit of body wt. rather than per unit of liver wt.

The results will be discussed in relation to hepatic changes in enzyme activities after thyroxine treatment (Murad & Freedland, 1967).

Murad, S. & Freedland, R. A. (1967). *Proc. Soc. exper. Biol. Med.* **124**, 1176.

Ross, B. D., Hems, R. & Krebs, H. A. (1967). *Biochem. J.* **102**, 942.

Control of Citrate Formation in Rat Liver by the Nicotinamide–Adenine Dinucleotide Redox State

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Studies with rat liver perfused with medium containing albumin and alanine (Williamson, Kreisberg & Felts, 1966) and with rat liver *in vivo* after glucagon infusion (Williamson, 1967), demonstrated that increased gluconeogenesis associated with enhanced fatty acid availability was accompanied by increases of ketogenesis, NADH, NADPH and ratios of lactate/pyruvate, malate/oxaloacetate, and by a decrease of the ATP/ADP ratio. Enhanced oxidation of fatty acids by liver is considered to diminish flux through the citric acid cycle (Krebs, 1966). Shepherd & Garland (1966) have proposed that this effect is mediated by inhibition of citrate synthase by ATP.

In view of the lack of correlation between changes of ATP in the whole liver and the proposed mechanism for the control of citrate formation, we now report results with rat liver mitochondria incubated with malate, L(-)palmitoylcarnitine and fluorocitrate. Respiration was initiated by one of the following: glucose-hexokinase, valinomycin, uncouplers, or 2-methyl-1,4-naphthaquinone (K_3). Suitable combinations of these reagents with oligomycin and atractyloside allowed the rate of citrate formation to be studied as a function of: (1) respiratory rate; (2) endogenous ATP level; (3) pyridine nucleotide redox state; (4) acetyl CoA and fatty acyl-CoA levels, and (5) malate availability.

No positive correlation was observed between the rate of citrate formation and the intramitochondrial ATP level when the nicotinamide nucleotide redox state remained constant, with respiratory rate either a variant or an invariant parameter. However, a close correlation was obtained between the rate of citrate formation and the intramitochondrial NAD/NADH ratio. Citrate formation was appreciably favoured by increased intramitochondrial malate concentrations only at high NAD/NADH ratios. Filtration studies with [^{14}C]-malate showed that valinomycin with 2 to 10mM-K^+ was effective in increasing mitochondrial malate, which entered with K^+.

It is proposed that the rate of citrate formation is controlled primarily by the redox state of the intramitochondrial NAD system, which in turn determines the oxaloacetate availability. Ketogenesis and diminished citric acid cycle activity is thus a direction consequence of the increased redox state induced by enhanced oxidation of fatty acids.