Urate Synthesis in the Perfused Chicken Liver

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Livers of cockerels (3–6 weeks' old) were perfused with 200 ml of a 2:1 (v/v) mixture of Krebs–Ringer bicarbonate and whole chicken blood, to which 360 mg of D-glucose was added, by the method of Bickerstaffe *et al.* (1970).

After an initial high rate, endogenous urate output, measured by the method of Kalckar (1947), was found to decrease to a mean linear rate of 0.78 ± 0.08 (10) (mean $\pm$ S.E.M. of ten samples) mg/h per g of liver for periods up to 150 min. Output and accumulation within the liver, measured by the method of Bokori (1965), were not markedly affected by the addition of 5 mM-NH₄Cl, 5 mM-L-glutamine or 5 mM-glycine, either individually or as a mixture. The addition of xanthine (1.65 mM), however, was found to increase urate output by $298 \pm 34\%$ (4). Similarly, stimulations of $314 \pm 12\%$ (3), $303 \pm 6\%$ (5), $210 \pm 30\%$ (4) and $72 \pm 10\%$ (4) were caused by additions of inosine (1.00 mM), inosine 5-monophosphate (0.60 mM), 4-amino-5-imidazolecarboxamide ribotide (0.75 mM) and 5-phosphoribose 1-pyrophosphate (0.55 mM) respectively. All these stimulations were statistically significant.

Addition of 0.5×10^{-3} i.u. of insulin/ml of perfusion medium, every 30 min, increased urate output in the presence of 5 mM-NH₄Cl by 21% (25 and 18%), and additions of 5 mM-L-glutamine, 5 mM-glycine and 5 mM-L-glutamine + 5 mM-glycine to this system increased outputs by $62 \pm 4\%$ (4), 126% (130 and 122%) and $140 \pm 3\%$ (5) respectively. L-Aspartate produced no stimulation of urate output.

The mean [ATP]/[ADP] ratio *in vivo* (Adam, 1965) of the chicken liver, namely 0.25 ± 0.002 (8), was changed little by perfusion and is apparently not markedly affected by insulin addition. This ratio is very low compared with that of 2.50 ± 0.10 (3) found for rat liver *in vivo*. [Lactate]/[pyruvate] ratios (Hohorst, 1965; Bücher *et al.*, 1965) in the perfused chicken liver were found to vary between 6.5 and 10.5. Water content of the liver increased by less than 1% during 150 min perfusion.

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Studies on the Relationship between Concentrations of Hepatic Metabolites and Induction of Glycolytic Enzymes *in vivo*

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The time-course for the induction of the three key glycolytic enzymes of rat liver, glucokinase, phosphofructokinase and liver-type pyruvate kinase, was investigated by re-feeding both diabetic and non-diabetic starved rats on protein diets supplemented with glucose, sucrose or glycerol and by injecting diabetic rats with insulin. The results show that when both glucokinase and liver-type pyruvate kinase are induced by the same conditions the time-course of their induction varies in such a way that substrate entering glycolysis at the hexose level induces glucokinase before liver-type pyruvate kinase, whereas substrates entering at the triose level induce liver-type pyruvate kinase before glucokinase. Whereas glucokinase induction requires insulin, liver-type pyruvate kinase may be induced in the diabetic animal, but only by substrates entering glycolysis at the triose level.

The activity of phosphofructokinase varies much less than that of the other two enzymes, the changes detected being of the same order as those found for the constitutive enzymes hexokinase and muscle-type pyruvate kinase. Thus it decreases slightly in starvation, returns towards normal on re-feeding and is not appreciably affected by diabetes.

Contrary to the concept of insulin as the inducer of the three key glycolytic enzymes (Weber *et al.*, 1966a,b), our results suggest that their induction is separately controlled. Although glucokinase is probably induced by insulin, the behaviour of liver-type pyruvate kinase is more readily explained as an independent induction by an intermediate at or below the triose level (Bailey *et al.*, 1968; Sillero *et al.*, 1969). Therefore hepatic metabolite concentrations were determined under conditions identical with those in the induction experiments and the results examined for correlations between these metabolite concentrations and enzyme activities. Glucokinase induction is accompanied by an increase in anhydride-bound phosphate and in the state of oxidation of the cytosol. Changes in the activity of liver-type pyruvate kinase