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

are apparently correlated with changes in dihydroxyacetone phosphate concentration. In non-diabetic rats they are both decreased in starvation and increased 48 h after re-feeding with glucose, glycerol or sucrose. Further, in diabetes the activity of liver-type pyruvate kinase and dihydroxyacetone phosphate concentration are decreased, and in this state both are raised by treatment with insulin and by re-feeding with sucrose or glycerol but not with glucose. However, no causal relationship has been established.

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The Effect on the Activities of some Hepatic Enzymes of Weaning Rats on to a Diet High in Sucrose Content

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There is a marked increase in hepatic lipogenesis and the activities of enzymes associated with lipogenesis on weaning rats from the high-fat-content milk diet of suckling to the high-carbohydrate (starch)-content laboratory diet (Taylor *et al.*, 1967; Lockwood *et al.*, 1970), Hepatic lipogenesis is also considerably higher in rats fed on sucrose rather than on starch-containing diets (Bailey *et al.*, 1968). To investigate further the involvement of diet in the post-weaning increase in lipogenesis we have studied the effect on the activity of some enzymes involved in carbohydrate conversion into fatty acids of weaning female rats on to a diet high in sucrose content.

Fructokinase (EC 2.7.1.4) activity increased after weaning (at 21 days of age) to a maximum at 40–45 days and then fell to pre-weaning values by the end of the experiment (70 days of age), and feeding with sucrose had little effect on the enzyme activity. Phosphofructokinase (EC 2.7.1.11) and fructose 1,6-diphosphatase (EC 3.1.3.11) activities varied little during the period studied and dietary sucrose had little effect on the activity of either enzyme. Aldolase (EC 4.1.2.13) activity was constant between 21 days and 40 days of age, rose to a maximum at 50 days and then fell to 70 days of age. Feeding with sucrose caused slightly elevated aldolase activities at all ages studied. Pyruvate kinase (EC 2.7.1.40) activity rose steadily during the experiment to a maximum at about 50–60 days of age, and activities in the livers

of sucrose-fed animals were always higher than those of controls. ATP citrate lyase (EC 4.1.3.8) and 'malic' enzyme (EC 1.1.1.40) increased in activity after weaning to a maximum at 35–40 days of age and then fell to the end of the experiment, and feeding with sucrose caused elevated activities at all ages studied. Extramitochondrial aconitate hydratase (EC 4.2.1.3) and NADP-dependent isocitrate dehydrogenase (EC 1.1.1.42) activities fell after weaning until 25–30 days of age and then rose throughout the rest of the experimental period. From 30 days of age the activities of both these enzymes were lowered by feeding with sucrose. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) activity rose rapidly after weaning to 40 days of age and then remained constant for the remainder of the experimental period. Feeding with sucrose increased this enzyme activity at all ages studied.

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Changes in the Activities of some Kidney Enzymes during Development of the Rat

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Although changes in the activities of many hepatic enzymes during development of the rat are well documented, little is known about the developmental changes that occur in the activity of rat kidney enzymes (for review see Greengard, 1971). We have measured the postnatal changes that occur in the activities of the following kidney enzymes: hexokinase (EC 2.7.1.1), pyruvate kinase (EC 2.7.1.40), 'malic' enzyme (EC 1.1.1.40), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), extramitochondrial NADP-dependent isocitrate dehydrogenase (EC 1.1.1.42), extramitochondrial aconitate hydratase (EC 4.2.1.3), ATP citrate lyase (EC 4.1.3.8) and fructose 1,6-diphosphatase (EC 3.1.3.11). We have also measured developmental changes in the incorporation of [^{14}C]acetate into lipid by kidney homogenates. The experiments were carried out with both male and female rats weaned at 21 days of age, and unless otherwise stated similar results were obtained with both sexes.

Hexokinase activity increases after birth to a maximum at 30 days of age, thereafter falling to reach adult values by 50 days of age. These results extend