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Lipid Metabolism in Experimental Cholestasis

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Disturbances in lipid metabolism have been reported in human and experimental obstructive jaundice. Thus human studies have shown elevated concentrations of plasma free fatty acids (Mortiaux & Dawson, 1961), serum triglycerides (Mendenhall & Mortiaux, 1961) and cholesterol (Man, Kartin, Durlacher & Peters, 1945), whereas in animal experiments elevated concentrations of cholesterol (Byers, Freedman & Michaelis, 1951) and abolition of the negative-feedback regulation of cholesterol biosynthesis (Katterman & Creutzfeldt, 1970) have been described. The purpose of the present investigation was to study lipid and ketone-body metabolism in the rat during experimental cholestasis.

Bile-duct ligation or sham operations were performed on anaesthetized rats and the animals were killed 6–9 days later. All rats were starved for 48h before being killed. Blood was collected from one group for determinations of triglycerides, free fatty acids, cholesterol and bilirubin and enzymic analysis of blood metabolites. The mean bilirubin concentration in the ligated animals was 9mg/100ml.

Another group of animals were killed by cervical dislocation, and the livers were freeze-clamped between clamps cooled in liquid N_2 and metabolites were determined in the deproteinized liver extracts by enzymic methods.

The most striking change noted was a fall in blood and liver ketone-body concentrations to 31 and 28% respectively compared with the corresponding values for the sham-operated rats after 9 days of biliary stasis. This was accompanied by a 73% decrease in hepatic acetyl-CoA concentration. The [3-hydroxybutyrate]/[acetoacetate] ratio fell from 2.7 to 1.1 and this was associated with a fall in the [glutamate]/[2-oxoglutarate][NH_4^+] ratio consistent with a more oxidized mitochondrial [free NAD^+]/[free NADH] ratio (Williamson, Lund & Krebs, 1967). The [lactate]/[pyruvate] ratios remained unchanged. Concentrations of plasma free fatty acids were normal or only slightly lower than in the controls, whereas those of plasma triglycerides and serum cholesterol were increased (146 and 200% respectively).

These results suggest a decrease in the β-oxidation of fatty acids and a stimulation of cholesterol and triglyceride synthesis. Possible mechanisms for these metabolic alterations will be discussed.

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An Explanation for the Lowering of Blood Ketone-Body Concentrations in Starved Rats during Short-Term Exercise

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The fall in blood ketone-body concentrations (45%) in starved rats during short-term exercise was ascribed by Drury, Wick & MacKay (1941) to increased peripheral utilization of ketone bodies. However, electrical stimulation of isolated perfused rat skeletal muscle does not increase ketone-body uptake (Houghton & Ruderman, 1970). Short-term severe exercise is also characterized by a rise in circulating lactate concentration (up to about 10mM), and as lactate can have an anti-lipolytic action (Bjorntrop, 1966) the effect of infusion of L(+)-lactic acid into resting starved (48h) rats was investigated. A concentration of about 7mM was reached in 10min and maintained for a further 20min. Blood ketone-body concentrations fell from 4.51 ± 1.51mM (mean $\pm$ S.D., five observations) to 2.29 ± 0.55mM by 10min and to 1.03 ± 0.41mM at 30min. There was a concomitant decrease in the plasma free fatty acid concentration from 1.40 ± 0.23 μequiv./ml to 0.74 ± 0.18 μequiv./ml after 30min infusion. On cessation of infusion there was a rise in the free fatty acid and ketone-body concentrations.

The results suggest that the fall in the concentration of blood ketone bodies during short-term exercise is mainly due to an inhibition of free fatty acid release from adipose tissue and perhaps also to a direct anti-ketogenic effect of lactate on the liver.

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The Effects of Physical Training on the Metabolic Response to Short-Term Severe Exercise in the Rat

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There have been many investigations of the effects of physical training on various enzyme activities in skeletal muscle (see Holloszy, 1967; Holloszy, Oscai, Don & Molé, 1970), but no studies have been made on metabolite concentrations in blood and liver. In the present experiments a 6-week physical training programme of increasing periods of running was used to study whether there was any metabolic adaptation to exercise accompanying the reported changes in the activities of muscle enzymes. Control rats (48h-starved females) immediately after running for 10min at 27m/min showed a rise in blood lactate concentration from 2.01 ± 1.09mM (mean±S.D., eight observations) to 9.51 ± 2.26mM and falls in the concentrations of total blood ketone bodies from 3.39 ± 0.99mM to 1.92 ± 0.65mM and of plasma free fatty acids from 1.49 ± 0.23mequiv./l to 1.17 ± 0.10mequiv./l. In contrast, trained rats showed only a slight increase in blood lactate concentration from 1.53 ± 0.65mM to 3.54 ± 1.77mM and a fall in that of total blood ketone bodies from 2.18 ± 1.15mM to 1.21 ± 0.30mM, but no significant change in that of plasma free fatty acids. These changes in metabolite concentrations in blood were paralleled by similar changes in the liver in both control and trained groups.

The hepatic concentration of alanine rose from $0.28\pm0.13\mu$mol/g wet wt. to $2.32\pm0.42\mu$mol/g in the control group, and there were simultaneous increases in aspartate from $0.66\pm0.11\mu$mol/g to $2.15\pm0.38\mu$mol/g and in malate from 0.24 ± 0.07 μmol/g to $0.82\pm0.24\mu$mol/g. In the trained group these increases were of smaller magnitude.

There was a 40% fall in hepatic ATP concentration in the untrained rats after exercise, whereas this decrease was 25% in the trained rats; the size of the total adenine nucleotide pool did not alter in either group.

Both groups of animals showed comparable increases in phosphorylated gluconeogenic intermediates, but the trained rats had higher glycogen and glucose concentrations after 10min of exercise.

All the changes in hepatic metabolite concentrations in untrained rats except the fall in that of ATP could be simulated by the intravenous infusion of L(+)-lactic acid into 48h-starved resting rats (see also Houghton, Hawkins, Williamson & Krebs, 1971).

It is concluded that the smaller changes in hepatic metabolite concentrations occurring in trained rats are mainly due to the lower circulating concentrations of blood lactate in response to exercise. Possible reasons for the fall in hepatic ATP concentration after short-term exercise will be discussed.

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Penetration of Perfused Rat Liver by Exogenous Amyloglucosidase

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Attempts have been made to alleviate certain types of glycogen-storage disease by intravenous administration of a glycogenolytic enzyme (Baudhuin, Hers & Loeb, 1964; Hug & Shubert, 1967; Lauer, Mascarinas, Racela, Diehl & Brown, 1968; Fernandes & Huijing, 1968). It was therefore decided to investigate the penetration of enzyme into liver. In the present experiments isolated rat liver was perfused by the technique of Hems, Ross, Berry & Krebs (1966) with medium containing *Aspergillus niger* amyloglucosidase (EC 3.2.1.3).

To enable accurate assessment of enzyme uptake it was necessary to ensure that all enzyme-containing perfusion fluid was removed at the end of the experiment. This was most satisfactorily achieved by using defibrinated (Baron & Roberts, 1963) whole rat blood instead of aged human erythrocytes, thus permitting removal of perfusion fluid with saline.

After perfusion for up to 4h with 30–50ml of medium containing 30–250mg of amyloglucosidase, the decrease in enzyme concentration in the medium was negligible. Hence the amyloglucosidase was rapidly destroyed neither in the perfusion

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