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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 ± 1.09 mM (mean $\pm$ S.D., eight observations) to 9.51 ± 2.26 mM and falls in the concentrations of total blood ketone bodies from 3.39 ± 0.99 mM to 1.92 ± 0.65 mM and of plasma free fatty acids from 1.49 ± 0.23 mequiv./l to 1.17 ± 0.10 mequiv./l. In contrast, trained rats showed only a slight increase in blood lactate concentration from 1.53 ± 0.65 mM to 3.54 ± 1.77 mM and a fall in that of total blood ketone bodies from 2.18 ± 1.15 mM to 1.21 ± 0.30 mM, 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 ± 0.13 μ mol/g wet wt. to 2.32 ± 0.42 μ mol/g in the control group, and there were simultaneous increases in aspartate from 0.66 ± 0.11 μ mol/g to 2.15 ± 0.38 μ mol/g and in malate from 0.24 ± 0.07 μ mol/g to 0.82 ± 0.24 μ 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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medium nor by the liver. The experiments with amyloglucosidase showed no difference in glycogen concentration in the liver nor in glucose release into the medium when compared with control perfusions.

Nevertheless there was penetration of liver tissue by circulating amyloglucosidase during perfusion corresponding to 0.03–0.4 mg of enzyme/g wet wt. of liver (corrected for endogenous glucosidase activity). The concentration in liver was never greater than that in the medium.

When amyloglucosidase was administered intravenously to intact rats, a major proportion of the enzyme found in the liver was identified in a mitochondrial-lysosomal fraction prepared by the procedure of Lloyd (1969a), confirming the results of Lloyd (1969b). After perfusion with amyloglucosidase increased glucosidase activity was also associated with a mitochondrial-lysosomal fraction. Although penetration of liver lysosomes by amyloglucosidase apparently occurred during perfusion under the present conditions, the extent of this process was less than *in vivo*. This may be due to the absence of cofactors or to lysosomal damage in the isolated liver.

Attempts to separate hepatocytes from Kupffer cells in an effort to identify the involvement of each in enzyme uptake *in vivo* have been made.

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Liposomes as Carriers of Enzymes or Drugs: a New Approach to the Treatment of Storage Diseases

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Patients with disorders in which a specific enzyme activity is absent from one or more tissues have in a few cases been treated by direct administration of an enzyme designed to remove undesirable accumulation products (Baudhuin, Hers & Loeb, 1964;

Fernandes & Huijing, 1968; Kissel, Lamazche & Royer, 1968). Such a therapeutic approach, however, involves possible immunological response arising from the foreign protein and problems related to undesirability of the presence of some enzymes in the circulation and in directing the given protein to a particular tissue.

It seemed possible that some of the difficulties mentioned could be avoided by entrapping relevant enzymes within liposomes. Liposomes (Bangham, 1968) consist of a series of concentric bilayers of lipids alternating with aqueous compartments within which soluble substances can be entrapped (Sessa & Weissman, 1968).

The present experiments describe conditions for the entrapment of *Aspergillus niger* amyloglucosidase (EC 3.2.1.3) [which splits (1→4)- and (1→6)-bonds of glycogen] and ¹³¹I-labelled albumin into liposomes composed of phosphatidylcholine, cholesterol and dicetyl phosphate. An entrapment of 4–10% of the proteins used was achieved (Gregoriadis, Leathwood & Ryman, 1971). More than 60% of protein-containing liposomes (with a lipid content of 2.5 mg) are eliminated within 10 min after their intravenous administration to rats. Experiments with ¹³¹I-labelled albumin entrapped into [³H]cholesterol liposomes suggest that liposomes remain intact during their circulation and indicate that there is no measurable leakage of ¹³¹I-labelled albumin. Most of the radioactivity removed from plasma is recovered in liver (and to a smaller extent in spleen) and it reaches a maximum (56% of that injected) during the first 15 min. There is a rapid decrease in liver radioactivity thereafter. After subcellular fractionation of the liver it appears that the mitochondrial-lysosomal fraction and cytosol are the major sites of liposomal recovery. Further preliminary fractionations on sucrose density gradients suggest that the liposomes may be associated with lysosomes of decreased density. Radioautography of the liver after injection of [³H]cholesterol liposomes reveals that both parenchymal and Kupffer cells participate in the uptake of liposomes. Liposomes appear to be a promising vehicle for the direction of enzymes and drugs to the liver and spleen.

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