Inhibition of Glucose Utilization by Perfusion with Chylomicrons in Rat Heart

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In rat hearts perfused with medium containing glucose and insulin the addition of palmitate in combination with bovine plasma albumin inhibits the phosphorylation of glucose, increases the intracellular concentrations of acetyl-CoA, citrate and glucose 6-phosphate and lowers that of CoA (thiol). The effects of palmitate have been attributed to oxidation of the fatty acid leading through changes in the concentrations of acetyl-CoA and CoA (thiol) to accumulation of citrate, inhibition of phosphofructokinase by citrate, accumulation of glucose 6-phosphate and inhibition of hexokinase by glucose 6-phosphate (Randle *et al.* 1966). Recent investigations by Crass & Meng (1966), Scheuer & Olson (1967) and Enser, Kunz, Borensztajn, Opie & Robinson (1967) have shown that chylomicrons may furnish fatty acids for oxidation in the perfused heart. In the present report the effects of esterified fatty acids in chylomicrons on glucose utilization and intracellular concentration of glucose 6-phosphate, citrate, acetyl-CoA and CoA (thiol) have been investigated. These studies were done in the absence of albumin, since the question has been raised as to whether albumin itself or some factor associated with it may be responsible for some of the changes induced by albumin-bound palmitate (e.g. Kipnis, 1966). Moreover in the presence of albumin the hydrolysis of chylomicrons by lipoprotein lipase in the perfusion medium may be stimulated (Enser *et al.* 1967) and albumin-bound free fatty acids formed.

Chylomicrons were isolated from thoracic-duct lymph of rats given corn oil as described by Ontko & Zilversmit (1967) and suspended and stored at 0° in 0.9% NaCl. Chylomicrons prepared by this procedure are essentially free of unesterified fatty acids (less than 0.04% of total fatty acids). Lipid content was assayed by drying to constant weight over P₂O₅ *in vacuo*. For perfusion the chylomicron suspension was added directly to the perfusion medium (less than 100 µl. of chylomicron suspension/10 ml. of medium) yielding a final concentration of 1.2 mg. of dry lipid/ml. Hearts from fed rats were prepared for perfusion as described by Newsholme & Randle (1964), except for omission of the injection with heparin. Oxygen consumption was measured in hearts perfused by drip-through as described by Randle, Newsholme & Garland (1964). Glucose uptake was calculated from measurements of the

glucose concentration in the medium for hearts perfused in a micro recirculation apparatus (England & Randle, 1967) by using hexokinase and glucose 6-phosphate dehydrogenase for spectrophotometric assay of glucose. Extracts of rat heart for assay of glucose 6-phosphate, citrate, CoA (thiol) and acetyl-CoA were prepared as described by Garland & Randle (1964). Glucose 6-phosphate was assayed spectrophotometrically with glucose 6-phosphate dehydrogenase, and citrate was assayed with citrate lyase and NAD-specific malate dehydrogenase (Garland & Randle, 1964). CoA (thiol) was assayed fluorimetrically with 2-oxoglutarate dehydrogenase (a gift of Dr R. M. Denton) by the method of Garland, Shepherd & Yates (1966). For assay of acetyl-CoA, the CoA (thiol) in extracts was removed either with 2-oxoglutarate dehydrogenase (see above) or by treatment with *N*-ethylmaleimide followed by 2-mercaptoethanol. Acetyl-CoA was then assayed as CoA (thiol) liberated by addition of oxaloacetate and citrate synthase.

The rate of oxygen consumption of hearts perfused for 20 min. with medium containing glucose (1 mg./ml.) and insulin (0.05 unit/ml.) was 1137 ± 48 µmoles of O₂/g. dry wt./hr. (mean $\pm$ s.e.m. for four hearts). Perfusion with chylomicrons suspended in the same medium led to a significant increase in oxygen consumption within 5 min. and the overall consumption during 20 min. of perfusion for four hearts was increased to 1405 ± 18 µmoles of O₂/g. dry wt./hr. ($P < 0.001$). The effect of chylomicrons on glucose uptake was measured in hearts perfused with medium containing glucose (2.5 mg./ml.) and insulin (0.05 unit/ml.). The control rate of glucose uptake was determined in an initial 10 min. period of perfusion; chylomicrons were then added and measurements continued for a further 50 min. (see Fig. 1 for results of typical experiments). Glucose uptake was decreased by the chylomicrons from a control value of 450 ± 10 to 262 ± 19 µmoles of glucose/g. dry wt./hr. (mean $\pm$ s.e.m. for three hearts, $P < 0.001$). In hearts perfused with medium containing glucose (2.5 mg./ml.) and insulin (0.05 unit/ml.) chylomicrons increased the concentration of glucose 6-phosphate from 1.9 ± 0.06 to 2.5 ± 0.1 µmoles/g. dry wt. ($P < 0.01$; six hearts in each group), increased that of citrate from 1.6 ± 0.17 to 2.5 ± 0.17 µmoles/g. dry wt. ($P < 0.01$; six hearts in each group), increased that of acetyl-CoA from $17 \pm$

Fig. 1. Effect of chylomicrons on glucose uptake in the perfused rat heart. Hearts were perfused for 5 min. by drip-through and then switched to recirculation with 8 ml. of medium containing glucose (2.5 mg./ml.) and insulin (0.05 unit/ml.). Samples of perfusion medium were taken for glucose analysis during recirculation at the times shown. (a) No chylomicrons added; (b) chylomicrons added after 10 min. of perfusion as indicated by the arrow; the broken line indicates the expected slope of glucose disappearance in the absence of chylomicrons.

2.2 to $45 \pm 3.8 \mu\text{moles/g. dry wt.}$ ($P < 0.002$; four hearts in each group) and decreased that of CoA (thiol) from 243 ± 21 to $147 \pm 17 \mu\text{moles/g. dry wt.}$ ($P < 0.01$; five hearts in each group). The acetyl-CoA/CoA concentration ratio was thus increased by chylomicrons from 0.07 to 0.31.

These changes in glucose uptake, oxygen consumption and glucose 6-phosphate, citrate, acetyl-CoA and CoA (thiol) concentrations in the heart with chylomicrons are similar to those previously described with albumin-bound palmitate (Newsholme & Randle, 1964; Garland & Randle, 1964; Challoner & Steinberg, 1966). These findings are consistent with the views that chylomicron fatty acids may be oxidized by the heart and that effects of albumin-bound palmitate are due to

palmitate oxidation and not to albumin. Scheuer & Olson (1967) and Enser *et al.* (1967) observed no increase in oxygen consumption in hearts perfused with chylomicrons, but in their perfusions glucose was not present in the medium.

In the present experiments perfusions were made with chylomicrons that were substantially free of unesterified fatty acids and without the addition of albumin. Therefore the effects observed during perfusion with chylomicrons may be attributed to esterified fatty acids and not, for example, to free fatty acids in the added chylomicrons or to free fatty acids released to albumin by circulating lipoprotein lipase (cf. Enser *et al.* 1967). These findings suggest that esterified fatty acids circulating as chylomicrons, in addition to plasma non-esterified fatty acids and muscle glyceride fatty acids, may have a controlling influence on myocardial carbohydrate metabolism *in vivo*.

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