After incubation, the triacylglycerol-rich lipoproteins seen under the transmission microscope appeared as partially degraded structures with atypical, disoid, flattened and lamellar forms. As shown in Table 1, heparin produced a significant increment in the hydrolysis of $\text{H}^-$labelled esterified fatty acids and $\text{H}^-$labelled acylglycerol from the $\text{H}^-$ and $\text{H}^-$labelled triacylglycerol-rich lipoproteins in the medium. A significant proportion of the hydrolysed labelled lipids appeared incorporated into the tissue lipids at the end of incubation. Most of the $\text{H}^-$labelled fatty acids appeared in their esterified form, whereas the $\text{H}^-$ taken up by the tissue appeared distributed in unesterified fatty acids, esterified fatty acids and acylglycerol.

The present results show that adipose tissue in situ is able to utilize not only the fatty acids but also the glycerol moiety released by the action of lipoprotein lipase on the lipoprotein triacylglycerol. This effect is quantitatively small but effective, agreeing with the reported ability of adipose tissue to metabolize glycerol (Chaves & Herrera, 1978; Herrera & Ayanz, 1972). It may be significant in situations of hyperlipidaemia in the presence of augmented adipose-tissue lipoprotein lipase and glycerokinase activities, as in obesity (Rath et al., 1974; Treble & Mayer, 1963).

This study was supported in part by a grant from the Comisi6n Asesora de Investigaci6n Cientifica y T6cnica de Spain.


Methionine metabolism via the transamination pathway in rat liver

GEOFFREY LIVESEY and PATRICIA LUND
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

The substrate specificity of rat-liver leucine (methionine) transaminase (Ikeda et al., 1976) and bacterial leucine dehydrogenase (Livesey & Lund, 1980) suggested that some steps in the catabolism of methionine and branched-chain amino acids may share common enzymes. Transamination then decarboxylation of the 2-oxo acid products initiates the catalysis of these amino acids (see Krebs & Lund, 1977; Mitchell & Benevenga, 1978; Steele & Benevenga, 1978). The methionine-transamination pathway operates independently of the trans-sulphuration pathway (Mitchell et al., 1974). The latter value provides a crude estimate of the rate of methionine metabolism via the transamination pathway operates independently of the trans-sulphuration pathway (Mitchell & Benevenga, 1978). The enzymes of the transamination pathway have not, however, been identified.

The specificity of the methionine transaminase activity was investigated by using intact hepatocytes. The cells (25 mg wet wt.), isolated as described by Krebs et al. (1974) from 48-h-starved female Wistar rats, were incubated for 30 min in 2 ml of Krebs–Henseleit (1932) saline containing 1 mM-L-$\text{H}^-$[1-'*C]methionine was also inhibited by 1 mM-2-cyano-4-hydroxycinnamate, a specific inhibitor of the mitochondrial pyruvate carrier (Halestrap & Denton, 1974) did not affect the rate of transamination of 4-methylthio-2-oxo-$\text{H}^-$[1-'*C]butyrate at either the concentration or absence of 1 mM-methionine, but the rate of 14CO$_2$ formation was decreased by 90%. This also suggests that 4-methylthio-2-oxobutyrate enters mitochondria on the pyruvate carrier and thus explains, in part at least, the inhibition by this 2-oxo acid of gluconeogenesis from pyruvate in kidney (Krebs & de Gasquet, 1964) and isolated hepatocytes (G. Livesey, unpublished work). Metabolic 14CO$_2$ formation from 1 mM-L-$\text{H}^-$[1-'*C]methionine was also inhibited by 1 mM-2-cyano-4-hydroxycinnamate.

Metabolic 14CO$_2$ formation from 0.05 mM-methylthio-2-oxo-$\text{H}^-$[1-'*C]butyrate (and from 1 mM-L-$\text{H}^-$[1-'*C]methionine) was markedly inhibited by branched-chain 2-oxo acids (1 mM), possibly by competition for the pyruvate carrier, which is known also to transport 4-methyl-2-oxovalerate, the 2-oxo acid analogue of leucine (Williamson et al., 1979; G. Livesey, unpublished work). Substrate specificity of the decarboxylation reaction was therefore investigated by using a preparation containing disrupted mitochondria. A rat liver homogenate was prepared in 0.01 M-K$_2$HPO$_4$/KH$_2$PO$_4$, pH 7.2, containing 1% (v/v) Triton X-100 and freeze(liquid N$_2$)-thawed (at 30°C) three times. 4-Methyl-2-oxo-$\text{H}^-$[1-'*C]valerate (0.2 mM) and 4-methylthio-2-oxo-$\text{H}^-$[1-'*C]butyrate (1 mM) were decarboxylated by the preparation at rates of 60 and 29 pmol/µg per g fresh wt. of liver respectively (K$_m$ values were 10 and 160 µM respectively) during a 2–10 min period at 38°C when these substrates were incubated with the equivalent of 2.5 and 10 mg of liver respectively in 1.0 ml of 20 mM-K$_2$HPO$_4$/KH$_2$PO$_4$, pH 7.2, containing 1.8 mM-MgSO$_4$, 0.4 mM-thiamin pyrophosphate, 0.4 mM-coenzyme A, 2.8 mM-NAD$^+$ (i.e. conditions similar to those for the assay of branched-chain 2-oxo acid dehydrogenase complex (Parker & Randle, 1978)). Omission of either MgSO$_4$, thiamin pyro-
phosphate, coenzyme A or NAD decreased the rates of $^{14}$CO$_2$ formation. Coenzyme A-dependence suggests that the product is 3-methylthiopropionyl-CoA and not 3-methylthiophosphate (Steel & Benevenga, 1978). Decarboxylation of 0.05 mM-4-methylthio-2-oxo-1-(${\text{14C}})$butyrate was inhibited by 20-fold higher concentrations of pyruvate and the 2-oxo acid analogues of methionine, leucine, valine and isoleucine by 35, 80, 97, 98% respectively. When 5$\mu$M-4-methyl-2-oxo-1-(${\text{14C}})$valerate was the substrate, 20-fold higher concentrations of these 2-oxo acids inhibited the initial rate of $^{14}$CO$_2$ production by 18, 25, 85, 88 and 80% respectively. These data suggest that the branched-chain 2-oxo acid dehydrogenase complex is the enzyme that catalyses the decarboxylation of 4-methylthio-2-oxobutyrate in rat liver. Further evidence of this possibility was obtained for us. Purified bovine kidney branched-chain 2-oxo acid dehydrogenase complex (but not pig heart pyruvate dehydrogenase complex) oxidized 4-methylthio-2-oxobutyrate ($K_m$ 124 + 12.3$\mu$M (mean ± S.E.M.) (K. S. Lau & P. J. Randle, unpublished work; assay based on NAD$^+$ reduction (Parker & Randle, 1978)).


Inhibition of rat liver glucose 6-phosphatase by p-chloromercuribenzenesulphonate

BEHZAD VAKILI and MALCOLM BANNER
Department of Biochemistry, Chelsea College, Manresa Road, London SW3 6LX, U.K.

It has been proposed by Arion et al. (1975) that the glucose 6-phosphatase activity (EC 3.1.3.9) of rat liver microsomes has two components, a phosphohydrolase responsible for catalysis, located on the luminal surface of the microsomal membrane and a permease, located on the cytoplasmic surface controlling the access of substrate to the phosphohydrolase. An important role for thiol groups in the activity of this enzyme has been suggested largely on the basis of inhibition by thiol alkylating and mercaptide-forming reagents (Colilla & Nordlie, 1973). We report here the effects of such one reagent, pCMS*, on the enzyme activity.

Microsomes were prepared from the livers of 24 h-starved rats by conventional differential centrifugation. Enzyme assays were performed at 30°C in a final reaction volume of 1 ml containing 20 mM-glucose 6-phosphate, 40 mM-cacodylate/HCl, pH 6.5, and 10 mg of bovine serum albumin/ml. Activity was determined from the rate of release of $P_i$. All preparations used had latencies of at least 40-50% (Arion et al., 1972) and values for non-specific phosphatase of less than 5% (de Duve & Honegger, 1972).

Inhibition studies were carried out by preincubating microsomes with the indicated concentrations of pCMS at pH 6.5 and 30°C. Microsomes were used either without prior treatment ('intact' microsomes) or after incubation with 0.05% (w/v) Triton X-100, providing that the reaction with pCMS was terminated as described above (Fig. 1e).

In 'intact' microsomes, immediate inhibition was also approx. 60% of the initial activity but was followed by a time-dependent inhibition of the remainder of the activity (Figs. Ig and 1h). A similar pattern of inhibition was seen in 'intact' microsomes, but only at concentrations of pCMS above 0.3 mM (Fig. 1f) and suggests that these higher concentrations of pCMS may increase membrane permeability as well as directly

This inactivation was reversed when the enzyme was assayed in the presence of a final concentration of 0.05% (w/v) Triton X-100, providing that the reaction with pCMS was terminated as described above (Fig. 1e).

In 'intact' microsomes immediate inhibition was also approx. 60% of the initial activity but was followed by a time-dependent inhibition of the remainder of the activity (Figs. Ig and 1h). A similar pattern of inhibition was seen in 'intact' microsomes, but only at concentrations of pCMS above 0.3 mM (Fig. 1f) and suggests that these higher concentrations of pCMS may increase membrane permeability as well as directly

---

* Abbreviation: pCMS, p-chloromercuribenzenesulphonate.