Liver cells were incubated in the presence of 0.05 mM-phenylalanine and glucagon (concentration range 10^{-11} to 10^{-8} M). Fluxes were determined by the [4-1^4C]phenylalanine method (Fisher & Pogson, 1984a). Each point represents the mean of four independent observations.

Fig. 1. Correlation between phenylalanine hydroxylation and homogentisate oxidation in isolated liver cells

Liver cells were incubated in the presence of 0.05 mM-phenylalanine and glucagon (concentration range 10^{-11} to 10^{-8} M). Fluxes were determined by the [4-1^4C]phenylalanine method (Fisher & Pogson, 1984a). Each point represents the mean of four independent observations.

mg per h; means ± S.E.M. for three different liver cell preparations. The effect of hormonal treatments on flux through homogentisate oxidase have also been assessed. Fig. 1 shows the correlation between the glucagon-dependent stimulation of phenylalanine hydroxylation and the corresponding increase in homogentisate oxidation. The data are consistent with the suggestion that flux through homogentisate oxidase is controlled largely by substrate supply. In this case, homogentisate supply is increased as a result of enhanced conversion of phenylalanine to tyrosine, a consequence of phenylalanine hydroxylation (Donlon & Kaufman, 1978; Fisher & Pogson, 1984b). Long-term changes in tyrosine aminotransferase activity, brought about by incubation of liver cells with a combination of glucagon (10^{-5} M) and steroid (triamcinolone), also result in enhanced homogentisate oxidation (basal: 28.36 ± 1.15, stimulated: 35.57 ± 2.15 nmol tyrosine transaminated/mg per 4 h; basal: 20.65 ± 2.37, stimulated: 28.99 ± 2.51 nmol and homogentisate oxidized/mg per 4 h, means ± S.D. for a single experiment).

These data indicate that although homogentisate oxidase activity has been reported to be hormonally sensitive (Lin & Knox, 1958), it appears that under physiological conditions the major influences on this step of catabolism are alteration of either phenylalanine hydroxylation or tyrosine aminotransferase.

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3-Hydroxyisobutyrate dehydrogenase: an impurity in commercial 3-hydroxybutyrate dehydrogenase

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The enzymic determination of ketone bodies (3-hydroxybutyrate and acetoacetate) utilizes 3-hydroxybutyrate dehydrogenase (HBDH; EC 1.1.1.30) of bacterial origin (Mellanby & Williamson, 1974). In these laboratories HBDH from Rhodopseudomonas spheroides (Grade II, Boehringer Corp. Ltd.) is in routine use. As part of a study of branched-chain amino acid metabolism in skeletal muscle (reviewed Palmer et al., 1985), the ketogenic capacity of muscle provided with branched-chain amino acid substrates was assessed. Hemidiaphragms from 40 h-starved rats (for experimental details, see Palmer et al., 1984a,b) were incubated with 3mM-leucine, 3mM-valine or with no added substrate. After 2 h, HClO₄ was added to the incubation media and 3-hydroxybutyrate and acetoacetate were determined after KOH neutralization. Using HBDH of commercial origin (grade II, Boehringer), it appeared that both leucine and valine promoted 3-hydroxybutyrate production (Table 1). Whereas acetoacetate and (via the HBDH reaction) 3-hydroxybutyrate are products of leucine metabolism, valine is metabolised via succinyl-CoA and is not ketogenic. It must be presumed therefore that the anomalously large amount of 3-hydroxybutyrate produced on valine addition is not 3-hydroxybutyrate per se but an impurity. Oxidation of 3-methyl-2-oxobutanoate (the 2-oxo acid analogue of valine) by isolated hepatocytes also gives rise to a product that behaves as 3-hydroxybutyrate in the HBDH reaction (Lund, 1981). This impurity may be 3-hydroxyisobutyrate, an intermediate in the pathway of valine oxidation; 3-hydroxyisobutyrate derived from its CoA ester is converted to methylmalonate semialdehyde in the 3-hydroxyisobutyrate dehydrogenase (HIBDH; EC 1.1.1.31) reaction. Commercial HBDH (grade II, Boehringer) contains HIBDH activity: its activity with 3-hydroxyisobutyrate (synthesized by the method of Coon, 1966) and confirmed pure by n.m.r. as substrate was approx. 10% of that with 3-hydroxybutyrate as substrate (in the assay of Bergmeyer et al., 1967). The Kₘ for 3-hydroxyisobutyrate...
was 0.1 mm (at 0.5 mm-NAD+*) and the pH optimum of the HIBDH activity was pH 9. The elution profile of the HIBDH activity was pH 9. The elution profile of the HIBDH activity was pH 9. The elution profile of the HIBDH activity was pH 9.

**Table I.** Apparent production of 3-hydroxybutyrate by hemidiaphragms from 40 h-starved rats provided with 3 mm-leucine, 3 mm-valine or no added substrate

<table>
<thead>
<tr>
<th>Addition</th>
<th>n</th>
<th>A (μmol/2 h per g wet wt.)</th>
<th>B (μmol/2 h per g wet wt.)</th>
<th>C (μmol/2 h per g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No substrate</td>
<td>24</td>
<td>0.64 ± 0.1</td>
<td>0.10 ± 0.04**††</td>
<td>0.56 ± 0.1</td>
</tr>
<tr>
<td>3 mm-Leucine</td>
<td>12</td>
<td>1.38 ± 0.2***</td>
<td>0.21 ± 0.1***</td>
<td>1.36 ± 0.2***</td>
</tr>
<tr>
<td>3 mm-Valine</td>
<td>12</td>
<td>1.51 ± 0.1***</td>
<td>0.45 ± 0.1***</td>
<td>0.68 ± 0.1††</td>
</tr>
</tbody>
</table>

**Control of proteolysis in isolated rat hepatocytes**

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In starvation, amino acids must be degraded in order to provide the liver with carbon for glucose synthesis; under these conditions the nitrogen atoms are mainly converted into urea via the ornithine cycle. The control properties of these two metabolic pathways have been extensively studied in our laboratory (Groen et al., 1983; Meijer et al., 1985). In the rat, an important source of amino acids in starvation is intrahepatic protein, the degradation of which mainly occurs via macroautophagy. Little is known about the mechanism(s) by means of which this process is controlled except that amino acids and hormones (glucagon, insulin) are involved (for a review, see Grinde, 1985).

In studies on the control of intrahepatic proteolysis use has been made of the isolated perfused rat liver (e.g. Schworer & Mortimore, 1979) and of suspensions of isolated hepatocytes in flask incubations (e.g. Seglen et al., 1980). There appears to be a large discrepancy between these two preparations. Whereas in the perfused liver mixtures of amino acids at their plasma concentration are sufficient to give substantial inhibition of proteolysis (Schworer & Mortimore, 1979), in isolated hepatocytes incubated with high concentrations of pyruvate much higher amino acid concentrations are required (Seglen et al., 1980).

In our studies, we have used isolated rat hepatocytes, both in a perfusion system (Van der Meer & Tager, 1976) and in closed-flask incubations. In hepatocytes perfused with glucose and octanoate, 50–60% inhibition of proteolysis was obtained with a mixture of all amino acids, each of which was present at its concentration in the portal vein.