Table 1. N-Terminal amino acid sequences of cytosolic aspartate aminotransferases

<table>
<thead>
<tr>
<th>Source</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>AcThr-Ser-Pro-Ser-Ile-Phe-Val-Glu</td>
</tr>
<tr>
<td>Rat**</td>
<td>Ala-Pro-Pro-Ser-Phe-Ala-Glu</td>
</tr>
<tr>
<td>Pig**</td>
<td>Ala-Pro-Pro-Ser-Val-Phe-Ala-Glu</td>
</tr>
<tr>
<td>Ox**</td>
<td>Ala-Pro-Pro-Leu-Met-Glu</td>
</tr>
<tr>
<td>Human*</td>
<td>Ala-Pro-Pro-Ser-Val-Phe-Ala-Glu</td>
</tr>
<tr>
<td>Chicken*</td>
<td>AcAla-Ala-Ser-Ile-Phe-Ala-Ala</td>
</tr>
</tbody>
</table>

terminal sequence in the mitochondrial isoenzymes may be taken to show that this part of the protein plays a specific biological role, possibly in transport into mitochondria.


Biliary excretion of [14C]androstenedione metabolites in control and clofibrate-treated male rats

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On administration to rats, clofibrate has been shown to increase the activities of hepatic microsomal enzymes responsible for the metabolism of drugs (Lewis et al., 1974) and steroid hormones (Salvador et al., 1970; Einarsson et al., 1973, 1974) and to increase hepatic cytochrome P-450 (Gibson et al., 1983). In this communication we report on the effect of clofibrate pretreatment on the metabolism of androstenedione in adult male rats in vivo.

Two groups of male laboratory-strain Wistar rats, weighing 150–360g, had free access to a commercial pellet diet for 2 weeks. The food given to one group was supplemented with 0.3% (w/w) clofibrate. The drug was added to the diet dissolved in ethanol, which was allowed to evaporate at room temperature. The control diet was treated with ethanol only. After 14 days the animals were anaesthetized and the common bile duct was cannulated. The cannula was exited from the back of the neck into a plastic bottle, which was strapped to the animal’s side, thus avoiding the necessity for a restraining cage. Immediately after cannulation the animals were injected subcutaneously with 0.2μCi of [14C]androstenedione (sp. radioactivity 59Ci/mmol) in 0.5ml of ethanol. The bile was collected for 24h, and samples were taken for radioactivity measurements.

The androstenedione metabolites were extracted from the bile by the method of Shackleton & Whitney (1980), by passing the diluted bile through a Sep-Pak C<sub>18</sub> cartridge (Waters Associates) and eluting with methanol. Recovery of radioactivity was determined by counting samples of the eluate. This was then evaporated to dryness and dissolved in 4ml of methanol/chloroform (1:1, v/v) 0.01m with respect to NaCl. This was applied to a Sephadex LH-20 column (1.5cm × 45cm), prepared and eluted in the same solvent. The free steroid, glucuronide and sulphate fractions were collected. The glucuronide fractions were combined and hydrolysed with β-glucuronidase. The androstenedione fractions were collected. The glucuronide and sulphate conjugates were used for determining [14C]radioactivity in the excreted bile.

Table 1. Effect of clofibrate on the biliary excretion of [14C]androstenedione metabolites in male rats

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control animals (11) treated animals</th>
<th>Clofibrate-pretreated animals (11) treated animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile volume (ml/24h)</td>
<td>13.51 ± 0.66</td>
<td>14.20 ± 0.73*</td>
</tr>
<tr>
<td>Radioactivity in 24h</td>
<td>55.68 ± 4.09</td>
<td>51.38 ± 3.27**</td>
</tr>
<tr>
<td>Radioactivity in glucuronide fraction (% of total excreted)</td>
<td>79.14 ± 1.84</td>
<td>86.43 ± 1.17**</td>
</tr>
<tr>
<td>Radioactivity in sulphate fraction (% of total excreted)</td>
<td>19.88 ± 1.77</td>
<td>12.02 ± 0.84**</td>
</tr>
</tbody>
</table>

Results are expressed as means ± S.E.M. for the numbers of animals in parentheses. *Not significant; **P<0.01. Treatment of animals and extraction and separation of metabolites are described in the text.

1984
On hydrolysis and chromatographic separation of the \([^{14}C]\)androstenedione glucuronide fraction, it was found there were significant differences in the chromatographic profiles obtained from control and clofibrate-treated animals. The latter showed a marked increase in the formation of the more polar metabolites. As clofibrate pretreatment has previously been shown to alter the metabolism of steroid hormones in vitro and to increase the activity of some of the steroid hydroxylases in rat liver microsomal fractions (Einarrsson et al., 1973, 1974), we tentatively suggest that this increase is due to the enhanced formation of hydroxylated steroids in vivo. Positive identification of these polar metabolites, however, will require g.l.c.–mass spectrometry.

The clofibrate used in this study was generously given by I.C.I. (England).


Vol. 12

The effects of surgery and hepatic dysfunction on alanine metabolism in man

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The rate of disappearance of an intravenous alanine load has been used as a method of investigating alanine metabolism in man and, by inference, hepatic gluconeogenesis (Felig et al., 1969; Fernandes & Blom, 1974; Elia et al., 1980; Royle & Kettlewell, 1981). The results of Elia et al. (1980) suggest that many of the alterations in blood alanine concentrations found during starvation, surgery and diabetes may be related to changes in the rate of clearance of an alanine load. For example, the decrease in blood alanine observed after surgery was associated with an increased clearance of infused alanine, suggesting that an augmented hepatic uptake caused the hypoalaminemia. In the present study we have examined the effects of severe hepatic dysfunction and major abdominal surgery on the disposal of an intravenous alanine load in man.

Ten patients with severe obstructive jaundice (serum bilirubin 158–555 mmol/l) secondary to malignant tumours of the biliary tract, and ten patients with malignant tumours of the large bowel, but normal liver function, were studied in the resting state after an overnight fast. A further eight patients with malignant tumours of the bowel were also investigated during surgical resection of the tumour. All three groups of patients were comparable for age and body weight.

The intravenous alanine test was undertaken as described by Elia et al. (1980), except that 140 mmol of L-alanine was infused and blood samples were collected 10, 20, 30, 40, 50 and 60 min after completion of the alanine infusion. The blood samples were analysed in duplicate for glucose, lactate, pyruvate, alanine, non-esterified fatty acids and 3-hydroxybutyrate concentrations (Walsh et al., 1981). In the next eight patients undergoing surgery the alanine test was undertaken after 60 min of the operative procedure.

The blood alanine half-life after infusion was derived from a semi-logarithmic plot of alanine concentration versus time by using the line of best fit between 20 and 60 min. The distribution volume of alanine was calculated by extrapolating the line of best fit back to zero time. The results are expressed as mean values (±S.E.M.).

There were no significant differences between control blood alanine concentrations, alanine half-lives and alanine distribution volumes in patients with normal liver function and severe jaundice (232±24 mmol/l, 40.8±1.6 min, 42.1±1.0 litres, and 317±22 mmol/l, 40.2±3.0 min, 41.3±2.4 litres, respectively). The changes in circulating metabolites in response to the alanine load were similar in both groups of patients, with small increments in blood glucose, lactate and pyruvate values. Plasma non-esterified fatty acid concentrations declined progressively in both groups during the 60 min after alanine, probably as a result of insulin secretion (Royle & Kettlewell, 1981). Blood 3-hydroxybutyrate values were unchanged after alanine infusion.

In the eight patients studied during surgery, the control alanine concentration was 277±29 mmol/l, and the half-life was prolonged to 52.1±4.9 min, with an unchanged distribution volume of 38.4±3.0 litres. The pattern of changes in circulating metabolites during surgery was similar to that observed in the resting state, except for a much greater blood glucose and lactate response.

The distribution volumes and half-lives of alanine found during this study were slightly greater than those reported by Elia et al. (1980), but this may reflect the greater age of the subjects (mean age >70 years) and their pathology. The results demonstrate that severe hepatic dysfunction had no effect on the rate of removal of an intravenous alanine load. This finding is in contrast with previous data showing a decrease in alanine clearance in four subjects with hepatic cirrhosis (Elia et al., 1980).

The clearance of alanine (distribution volume × 0.693/half-life) is approx. 700 ml/min, which is very similar to the plasma flow through the liver. It is well recognized that when the clearance of a compound is high it is much more sensitive to changes in hepatic blood flow and relatively insensitive to the metabolic activity of the liver (Haberer et al., 1982). Thus the prolonged alanine half-life and decreased clearance found during surgery probably reflect the decline in hepatic blood flow that is found during surgery (Strunin, 1977). It is noteworthy that the much increased glucose response to alanine during surgery cannot be interpreted as an augmentation of gluconeogenesis. Indeed, we have observed similar changes in patients undergoing identical surgery without an alanine load (Hall et al., 1983).

In conclusion, the pharmacokinetics of alanine in man are likely to be strongly influenced by changes in hepatic blood flow and cannot be assumed to represent variations in the gluconeogenic capacity of the liver.
