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"First-pass" metabolism of paracetamol in rat liver

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Paracetamol was administered intraperitoneally or intravenously to male rats. Its 'availability' in the systemic circulation after intraperitoneal administration was only 34% of that after intravenous administration. This suggested a high "first-pass" metabolism of intraperitoneally administered drug, which was confirmed using the rat isolated perfused liver. The hepatic extraction ratio for paracetamol decreased with increasing concentrations of the drug in the perfusion fluid, suggesting that gastric emptying rate, by controlling the concentration of drug in the portal vein, could influence the amount of "first-pass" metabolism.

With some drugs, the area under a plasma concentration-time curve is proportional to the dose administered (Dost, 1958). For drugs such as propranolol (Shand, Nuckolls & Oates, 1970) and lignocaine (Boyes, Adams & Duce, 1970), the area under the curve describing oral or intraperitoneal (i.p.) administration is less than that following intravenous (i.v.) administration, although the drug is completely absorbed. The lower "availability" of these drugs following oral administration has been attributed to a significant amount of either uptake or metabolism, or both, at either the gut wall or the liver during the "first-pass" of the drug through these organs. A comparison of the area under the curve for paracetamol following intravenous or intraperitoneal administration to rats suggested a high "first-pass" clearance during absorption from the peritoneal cavity. Since drugs absorbed from the peritoneum must reach the systemic circulation via the portal venous system, it seemed likely that the liver was the site of this "first-pass" clearance. This was confirmed using the rat isolated perfused liver.

MATERIALS AND METHODS

Male Wistar rats (RPMS), 220-280 g, were injected with [^{3}H] paracetamol (generally labelled the New England Nuclear Corporation) (15 mg kg^{-1}, 6–10 μCi) either intraperitoneally or intravenously under light ether anaesthesia into the femoral vein (this did not affect the distribution or elimination of intraperitoneally administered paracetamol).

Blood samples (0·2 ml) were usually removed every 15 min from the tail vein for 1½–2½ h and added to 0·8 ml of an aqueous solution of paracetamol (1 mg ml^{-1}) as a carrier. Ethyl acetate (10 ml) was added and mixed vigorously for 2 min and the mixture centrifuged at 2000 rev min^{-1} for 10 min. 8 ml of the ethyl acetate extract was removed and evaporated to approximately 1 ml in a stream of N_2 and 10 ml of Instagel (Packard) added. Radioactivity was counted in a Packard liquid

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scintillation spectrometer. Recoveries of [^{3}H]paracetamol from aqueous solution and blood were $93 \pm 1{\cdot}4\%$ and $90 \pm 0{\cdot}7\%$ respectively by this method. The similarity of paracetamol concentrations in liver perfusates obtained by gas-liquid chromatography (Prescott, 1971) or this method confirmed its specificity.

Isolated perfused liver experiments

In a typical experiment the rat was anaesthetized with ether, the liver was perfused with medium (Hems, Ross & others, 1966) via the portal vein and was then removed and placed in the organ chamber as shown in Fig. 1. The apparatus was similar to that of Von Bahr, Alexanderson & others (1970) but with a second reservoir B

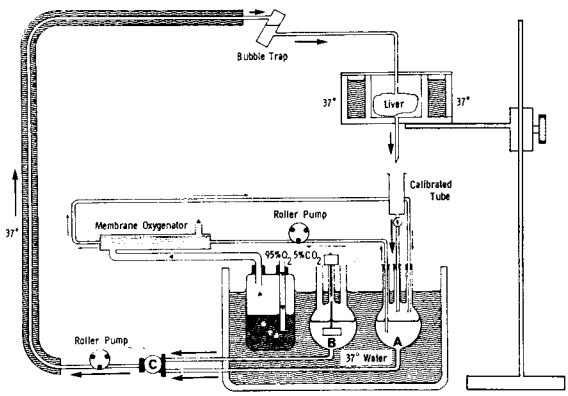

FIG. 1. Apparatus for the perfusion of rat isolated livers. For details see the text. Blood flow through the liver and the membrane oxygenator is indicated by the large and small arrows respectively. Gas flow is indicated by arrow heads.

containing perfusion medium equilibrated with drug. Blood is circulated from reservoir A through a 3-way stop-tap C via a roller pump and a bubble trap to the liver. The blood, after having passed through the liver, returned to reservoir A via a calibrated tube used to check the perfusion flow rate. Blood from reservoir A was oxygenated by circulation through a membrane oxygenator and returned to A. The liver was perfused for 30 min with a drug free perfusion medium from reservoir A to allow the liver to equilibrate. Then the drug equilibrated medium from reservoir B was allowed to pass through the liver at 20 ml min^{-1} and the effluent was collected in 5×1 min samples and analysed for total and ethyl acetate extractable radioactivity, using the method described. Individual extraction ratios (E.R.) were expressed as the mean of the five E.R. calculated in each experiment from:

$$\text{E.R.} = \frac{C_{in} - C_{out}}{C_{in}}$$

where C_{in} and C_{out} represent the concentration of the unchanged drug in the inflow and outflow respectively. After the first minute the E.R. was constant, suggesting that uptake and distribution equilibria had been established.

RESULTS AND DISCUSSION

Concentrations of paracetamol after intraperitoneal administration were significantly lower than after intravenous administration at all sampling times. The area under the concentration-time curve after intravenous administration of [^{3}H]paracetamol was significantly greater ($P<0{\cdot}005$ t-test) than after intraperitoneal administration (i.v. area $= 702 \pm 93$ μg ml^{-1} min^{-1}; $t_{\frac{1}{2}} = 28{\cdot}5 \pm 3{\cdot}2$ min, n $= 4$: i.p. area $= 240 \pm 7$ μg ml^{-1} min^{-1}; $t_{\frac{1}{2}} = 22{\cdot}8 \pm 4{\cdot}8$ min, n $= 4$). There was no significant difference in the plasma half-life of the drug between the routes. These two facts suggest that the blood concentration of the drug after intraperitoneal injection is the result of first-pass metabolism rather than delayed absorption. If it is assumed that all of the intraperitoneally administered paracetamol was absorbed, the results suggest a higher clearance of the paracetamol on its first passage through the liver to the systemic circulation. A comparison of the areas under the curve (measured by the trapezoid rule) following both routes of administration gives a measure of the availability of the paracetamol in the systemic circulation.

$$\text{Availability} = \frac{\text{Area under curve after i.p. injection}}{\text{Area under curve after i.v. injection}} = 0{\cdot}34 \quad \ldots\ldots \quad (1)$$

Gibaldi, Boyes & Feldman (1971) have derived the following equation to predict the extent of a first-pass effect for drugs:

$$f = \frac{\text{liver blood flow}}{\text{liver blood flow} + \left(\frac{\text{dose}}{\text{area}}\right)} \quad \ldots\ldots \quad (2)$$

where f is the proportion of the dose administered which reaches the systemic circulation and the area is the area under the curve following intraperitoneal injection. With a rat liver blood flow rate of 1·2 ml g^{-1} liver min^{-1} (Dobson & Jones, 1952) (liver weight $\simeq$ 8 g), together with the areas under the curves from the intraperitoneal route and substituting in equation (2), a value of f $= 0{\cdot}40$, is obtained which is in reasonable agreement with the experimentally observed value of 0·34.

The results of the studies using the rat isolated perfused liver (Table 1) confirmed a

Table 1. *Clearance of paracetamol by the rat isolated perfused liver.*

Paracetamol μg ml^{-1}	E.R.*	Paracetamol μg min^{-1}	Amount of metabolites in effluent† μg min^{-1}
1 (3)‡	0·52 ± ·01	10·4	2·72 ± 0·38
5 (3)	0·47 ± ·05	47	10·7 ± 0·4
10 (3)	0·36 ± ·08	72	15·8 ± 6·1
50 (3)	0·27 ± ·09	270	66·1 ± 15·1

*E.R. = Extraction Ratio $= \frac{C_{in} - C_{out}}{C_{in}}$

†The amount of metabolites was calculated from the non-extractable radioactivity in the perfusion effluent and expressed as paracetamol equivalents.

‡Number in parentheses equals number of experiments. Perfusion flow rate = 20 ml min^{-1}.

high clearance of paracetamol by the liver at low perfusion concentrations. However, when the perfusion concentration was increased from 1 to 50 μg ml^{-1}, the extraction ratio fell from 0·52 to 0·27 although the absolute amount of drug extracted increased. The extraction of paracetamol was also dependent on the rate of perfusion. In experiments with an inflow concentration of 5 μg ml^{-1}, when the perfusion flow rate was decreased from 20 to 10 ml min^{-1}, the extraction ratio increased from 0·47 to 0·75.

Recently, Heading, Nimmo & others (1973) have shown that gastric emptying controls the rate of absorption of paracetamol and consequently the peak plasma concentration achieved. Our findings suggest that it may also affect the amount of "first-pass" metabolism in the liver.

A fast rate of gastric emptying could give high concentrations of drug in the portal vein and thus a lower hepatic extraction ratio, thereby resulting in higher availability of the drug in the systemic circulation. The reverse would be true for a slow rate of gastric emptying. These considerations could be important with drugs known to have a high hepatic clearance.

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