KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). *J. Physiol. Lond.*, **136**, 249–262.
MANTOVANI, P. & VIZI, E. S. (1974). *J. Pharm. Pharmac.*, **26**, 461–462.
VAN NUETEN, J. M., GEIVERS, H., FONTAINE, J. & JANSSEN, P. A. J. (1973). *Archs int. Pharmacodyn. Thér.*, **203**, 411–414.
VAN NUETEN, J. M., JANSSEN, P. A. J. & FONTAINE, J. (1976.) *Life Sci.*, **18**, 803–810.

The enterohepatic circulation of oxazepam-*O*-glucuronide in guinea-pigs

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Previous studies from this laboratory have shown that oxazepam given intravenously is present as a glucuronide in the bile of guinea-pigs in relatively large amounts (Bertagni, Marcucci & others, 1972). Since in this species oxazepam disappears at a relatively low rate from the blood, the existence of an enterohepatic circulation was suggested (Mussini, Marcucci & others, 1972; Garattini, Mussini & others, 1973b). This hypothesis can now be supported.

Twelve male albino guinea-pigs (400 g) were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p. as a solution of 20 mg ml^{-1}). After a midline incision the cystic bile duct was ligated and the bile duct was exposed and cannulated with two polyethylene tubes. One tube (external diameter 0·96 mm) was placed in the duodenum through the bile duct, the other tube (external diameter 1·27 mm) was directed towards the liver to facilitate bile collection. The two tubes were passed between muscle and skin, fixed at the tissues and joined by means of an external by-pass. Thus, the bile circulation could be either maintained or interrupted.

Oxazepam-*O*-glucuronide was purified from rabbit urine by two chloroform extractions and two passages through activated charcoal columns, followed by thin-layer chromatography on silica gel preparative plates as described in detail elsewhere (Marcucci, Bianchi & others, 1975). The indentity of the glucuronide was confirmed by means of gas chromatography-mass spectrometry (Marcucci & others, 1975).

For the determination of free and conjugated oxazepam in the tissues, bile and urine, a g.c. method previously described by Bertagni & others (1972) was used. The conjugated oxazepam is expressed as oxazepam released after incubation with β-glucuronidase.

The animals had a good recovery from the surgical procedure and their apparent normal behaviour had advantages over that of anaesthetized animals as anaesthesia reduces biliary flow (Roberts & Plaa, 1967), biliary excretion of a variety of compounds (Klaassen, 1970), intestinal peristalsis and the progression of intestinal contents. The last point was illustrated using a Velva Glo-Red 103-115 suspension as an indicator of the intestinal peristalsis (De Feo, Piccinelli & Silvestrini, 1971) (250 mg kg^{-1}) injected through the duodenum of 4 of the animals. By this means the progression of the intestinal contents in animals with the artificial biliary circulation was shown to be more than 4 times greater than in similarly prepared anaesthetized animals. After the surgical procedure the animals were kept in individual restraining cages with food and water freely available. Urine was collected during the experiments which lasted 3 h, after which the guinea-pigs were killed and the tissues taken for analysis. The guinea-pigs were injected through the cannula entering the duodenum with oxazepam-*O*-glucuronide at a dose corresponding to 5 mg kg^{-1} of oxazepam. After the injection, the animals were divided into two groups each of 4 animals. In one group (A) the circulation of bile was interrupted. The bile was collected from the liver side while normal bile, obtained from untreated animals, was infused toward the intestine at the usual rate of production (0·1 ml min^{-1}). In the other group (B) the bile circulation was normal.

Table 1 shows that during the 3 h of the experiment the guinea-pigs in Group A excreted in the bile 32% of the injected oxazepam-*O*-glucuronide while only about 15% was excreted in the urine. The animals with an intact biliary circulation (Group B) eliminated in the urine twice as much oxazepam-*O*-glucuronide (about 30%) as Group A. Consistent with this difference in the amount of oxazepam-*O*-glucuronide excreted is the

Table 1. *Percentage of the dose and concentration of oxazepam and oxazepam glucuronide 3 h after intraduodenal injection of oxazepam glucuronide (5 mg kg^{-1}) in 2 groups (A and B) of 4 guinea-pigs each.*

Oxazepam in	A (±s.e.)	B (±s.e.)
Bile % of dose conjugated	31·7 ± 1·0	—
Urine % of dose conjugated	15·7 ± 2·8	29·1 ± 3·5*
Blood μg ml^{-1} — free	0·21 ± 0·03	0·8 ± 0·03*
	0·08 ± 0·03	0·2 ± 0·08
Brain μg g^{-1} — conjugated	0·98 ± 0·03	4·57 ± 0·2*
Adipose tissue μg g^{-1} free	0·41 ± 0·07	2·12 ± 0·3*

* $P<0·01$ with respect to Group A.

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higher concentration (about 4–5 times) of oxazepam in the blood, adipose tissue and brain of the animals with an intact biliary circulation compared with those having the bile fistula. The concentration of oxazepam-*O*-glucuronide in the blood of Groups A and B were however not significantly different, perhaps due to the high variability or to a high rate of clearance.

These results show that of animals injected intraduodenally with oxazepam-*O*-glucuronide those with an intact biliary circulation, after 3 h, have a concentration of oxazepam in the blood about four times higher than those with an interrupted biliary circulation. This demonstrates that oxazepam-*O*-glucuronide excreted in the bile must be rapidly absorbed through the intestine into the circulation. Since the blood concentrations of oxazepam are higher than those of its glucuronide it must be assumed that the glucuronide is largely hydrolysed to form oxazepam. This is supported by the finding of β-glucuronidase in the intestinal flora (Smith, 1966; 1970) and by the observation that the intestinal wall is also capable of hydrolysing oxazepam-*O*-glucuronide (E. Mussini, unpublished observations).

The lower concentrations of blood oxazepam in the animals with a biliary fistula also explain the reduced concentrations of oxazepam in the adipose tissue and in brain compared with the guinea-pigs with an intact biliary circulation. Previous studies have demonstrated a relation between blood oxazepam and brain oxazepam concentration (Garattini, Marcucci & others, 1973a). Furthermore the anticonvulsant activity depends on the concentration of brain oxazepam (Garattini & others, 1973a). That no oxazepam-*O*-glucuronide was detected in these tissues probably is a reflection of its low lipid solubility. Oxazepam-*O*-glucuronide is not only excreted in the bile but also in the urine of guinea-pigs; this is consistent with the observation that most of the compounds with a molecular weight between 350 and 450 (mol. wt of oxazepam-*O*-glucuronide is 463) can be eliminated by both routes (Hirom, Millburn & others, 1972). The higher urinary elimination of the glucuronide in guinea-pigs with an intact biliary circulation may be related to the compound's higher availability in the blood compared with the group of animals which lose oxazepam glucuronide through the biliary fistula.

That the enterohepatic circulation of oxazepam-*O*-glucuronide maintains sustained concentrations of oxazepam in blood may be extrapolated to other animal species. It has been found previously that the persistance of oxazepam in blood was proportional to the amount of oxazepam glucuronide excreted in the bile, being in mice > guinea-pigs > rats (Marcucci, Mussini & others, 1970). The importance of the enterohepatic circulation for oxazepam metabolism should be considered also when other benzodiazepines are administered, since in several cases the final metabolite is represented by oxazepam (Greenblatt & Shader, 1974). The variability in oxazepam blood concentration in man (Garattini & others, 1973a,b) may be at least partially ascribed to differences in the enterohepatic circulation.

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REFERENCES

BERTAGNI, P., MARCUCCI, F., MUSSINI, E. & GARATTINI, S. (1972). *J. pharm. Sci.*, **61**, 965–966.

DE FEO, G., PICCINELLI, D. & SILVESTRINI, B. (1971). *Farmaco ed. prat.*, **26**, 123–130.

GARATTINI, S., MARCUCCI, F., MUSSINI, E. & MORSELLI, P. L. (1973a). In: *Biological Effects of Drugs in Relation to their Plasma Concentrations*, p. 211–225. Editors: Davies, D. S. & Prichard, B. N. C., London: MacMillan.

GARATTINI, S., MUSSINI, E., MARCUCCI, F. & GUAITANI, A. (1973b). In: *The Benzodiazepines*, p. 75–97. Editors: Garattini, S., Mussini, E. & Randall, L. O.. New York: Raven Press.

GREENBLATT, D. J. & SHADER, R. I. (1974). *Benzodiazepines in Clinical Practice*. New York: Raven Press.

HIROM, P. C., MILLBURN, P., SMITH, R. L. & WILLIAMS, R. T. (1972). *Biochem. J.*, **129**, 1071–1077.

KLAASSEN, C. D. (1970) *J.Pharmac. exp. Ther.*, **175**, 289–300.

MARCUCCI, F. BIANCHI. R, AIROLDI, L., SALMONA, M., FANELLI, R., CHIABRANDO, C., FRIGERIO, A., MUSSINI, E. & GARATTINI, S. (1975). *J. Chromat.*, **107**, 285–293.

MARCUCCI, F., MUSSINI, E., FANELLI, R. & GARATTINI, S. (1970). *Biochem. Pharmac.*, **19**, 1847–1851.

MUSSINI, E., MARCUCCI, F., FANELLI, R. & GARATTINI, S. (1972). *Chem. Biol. Interact.*, **5**, 73–76.

ROBERTS, R. J. & PLAA, G. L. (1967). *Biochem. Pharmac.*, **16**, 327–835.

SMITH, R. L. (1966). *Prog. Drug Res.*, **9**, 299–360.

SMITH, R. L. (1970). *Proc. Eur. Soc. Study Drug Toxic.*, **11**, 19–32.