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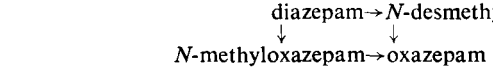
Metabolism of diazepam in isolated perfused liver of rat and mouse

SIR,—It was reported (Schwartz, Koechlin, & others, 1965; Schwartz, Bommer & and Vane, 1967) that in rats, dogs and man the major metabolic pathways of ^{3}H-labelled diazepam involved *N*-demethylation and C-3 hydroxylation, according to the following scheme:



We now confirm the formation of these metabolites by using the isolated perfused liver of rats and mice.

Male Sprague-Dawley rats (200 g) or male Swiss mice (21–24 g) were used as donors of blood and livers. The animals were kept on a standard diet (ALAL 56) and had food and water *ad libitum*. Diazepam or its metabolites were added to the perfusion fluid in concentrations of 50 μg/ml. Details of the technique for the perfusion of the isolated livers of rats and mice have been described elsewhere (Kvetina & Guaitani, 1968). At selected intervals, 2 ml amounts of the perfusion fluid were withdrawn from the circulation and extracted twice with 10 ml of ether. The combined ether extracts, of diazepam and its metabolites were evaporated to dryness.

The residue was dissolved in hexane and quantitatively transferred into a thin-layer plate of Silica Gel G. Pure standards of diazepam and its three metabolites were run alongside the sample extracts for identification of the compounds.

The plates were developed to 15 cm above the origin in glass tanks using the solvent system chloroform acetone, 90:10. After development, the plates were allowed to dry completely and then viewed under ultraviolet light (245–350 mμ) to identify the compounds on the plate.

The metabolites formed during the liver perfusion were further identified and verified by two-dimensional chromatography in at least three solvent system pairs (chloroform–heptane–ethanol (10:10:1), heptane–chloroform–acetic acid–ethanol (5:5:1:0·3), chloroform–acetone (90:10)).

The metabolite identification was reinforced by the colour reactions developed

when the plates were sprayed with the Dragendorff reagent modified by Munier & Macheboeuf (1951).

Comparison of the migration of the metabolites extracted from the liver perfusion fluid containing 50 μg/ml of diazepam with known standards indicated that there are no qualitative differences in the liver metabolism of diazepam between rats and mice.

The identified metabolites were: *N*-desmethyldiazepam, *N*-methyloxazepam and oxazepam.

Samples of the perfusion fluid at different times, showed that, at 5 min, only small quantities of *N*-desmethyldiazepam were present. By 15 min in the rat or by 30 min in the mouse, in addition to an increased amount of the above metabolite, *N*-methyloxazepam could also be identified. Oxazepam appeared at 30 min in the rat and at 90 min in the mouse liver perfusion fluid.

Studies on the metabolism of diazepam metabolites using the same technique of the perfused liver showed that the perfusion with *N*-desmethyldiazepam led to the formation of oxazepam both in rat and in mouse. The perfusion with *N*-methyloxazepam resulted in both species in the formation of oxazepam. In addition to oxazepam an unidentified metabolite was present only in the perfusion fluid coming from the liver of the mouse. The perfusion with oxazepam showed that there was no further formation of other metabolites. These results are summarized in Table 1.

TABLE 1. THE METABOLISM OF DIAZEPAM AND ITS METABOLITES IN ISOLATED PERFUSED LIVER OF RATS AND MICE

Compound added	Rf in chloroform–acetone (90:10)	Metabolites identified after 90 min
Diazepam	0·79	NDD, NMO, OX
N-Desmethyldiazepam (NDD)	0·41	OX
N-Methyloxazepam (NMO)	0·63	OX, unidentified*
Oxazepam (OX)	0·17	—

* Only for mouse liver.

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