Oxazepam Excretion by Chlordiazepoxide-^{14}C-Dosed Dogs

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The metabolism of chlordiazepoxide was studied in dogs using the ^{14}C-labeled drug. Following a single 26 mg./Kg. dose, oxazepam amounting to 1.1 per cent of the dose was found in urine and 1.3 per cent in feces. The oxazepam was identified by thin-layer chromatography. Chronic administration of chlordiazepoxide at a daily dose of 5 mg./Kg. for 9 weeks had no significant effect on the amount converted to oxazepam.

In previous reports on the metabolism in dogs of chlordiazepoxide (1) (7-chloro-2-methylamino-5-phenyl-3H-1, 4-benzodiazepine 4-oxide) and diazepam (2) (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), the authors concluded that the 3-position in the diazepine ring of chlordiazepoxide was not subject to metabolic attack. This would eliminate the possibility of oxazepam[1] (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one) formation from chlorodiazepoxide. However, evidence for the metabolic conversion of chlordiazepoxide to oxazepam in the dog in small but significant amounts has been obtained.

The conclusion is based on results of preliminary studies in which oxazepam was recovered from the urine of dogs fed "lactam" (7-chloro-1, 3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide), a major metabolite of chlordiazepoxide (1), and on chromatographic evidence obtained in studies in dogs fed chlordiazepoxide labeled with ^{14}C in the 2-position of the diazepine ring (specific activity 1.2 μc./mg.). The effect of chronic administration of chlordiazepoxide on the conversion to oxazepam was also investigated.

EXPERIMENTAL

Four dogs were given nonradioactive chlordiazepoxide in 5 mg./Kg. daily oral doses for 9 weeks followed by a final similar dose of chlordiazepoxide-^{14}C. In one phase of this study, two female dogs were given single 26 mg./Kg. oral doses of tagged chlordiazepoxide, and urine and feces were collected at intervals up to 48 hr. For thin-layer chromatography, the urine samples were exhaustively extracted with ethylene dichloride, and the aqueous residues were treated with β-glucuronidase at pH 5.5 for 48 hr. and then re-extracted with ethylene dichloride. Feces were also extracted with ethylene dichloride, but without glucuronidase treatment, since in a previous study (3) oxazepam was found to be excreted in feces unconjugated. The ethylene dichloride extracts were concentrated under vacuum to small volume and subjected to thin-layer and paper chromatography. In addition to the many solvent systems given in the references cited, the following were employed in thin-layer chromatography: (*a*) on Whatman Silica Gel SG 41: benzene–pyridine–acetic acid (80:5:2, v/v/v); chloroform–acetone–ethanol (8:5:5, v/v/v); and chloroform–acetone–ethanol (8:1:1, v/v/v); and (*b*) on MN-Kieselgel G-HR: *n*-butanol–*n*-butyl ether–acetic acid (4:8:1, v/v/v); and (*c*) on Silica Gel G (Merck, Darmstadt): heptane–chloroform–ethanol (5:5:1, v/v/v). After solvent development and air drying, the chromatograms were placed in contact with DuPont Cronex II medical X-ray film for 1–4 weeks, depending on the amount of radioactivity present. Radioactive areas on thin-layer plates as defined by radioautography were scraped into scintillation vials for assay on a Packard Tri-Carb liquid scintillation spectrometer using a dioxane "cocktail" (100 Gm. naphthalene, 10 Gm. POP, 250 mg. dimethyl POPOP, and 200 ml. methanol in 1 L. spectroquality 1,4-dioxane).

RESULTS AND DISCUSSION

Figure 1 is a radioautograph of a paper chromatogram of urine samples applied directly without prior treatment or extraction. The characteristic shape and location of oxazepam glucuronide can be seen duplicated in the urine samples from dogs dosed with diazepam and chlordiazepoxide, respectively. However, this area in the diazepam sample contains, in addition, the glucuronide of 3-hydroxy-diazepam (7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), and the same fraction in the chlordiazepoxide urine may also contain other glucuronides. Glucuronidase treatment of urine resulted in the disappearance of this glucuronide area, and gave rise to an ethylene dichloride extractable substance which when chromatogramed in all thin-layer systems cited under *Experimental* migrated identically with

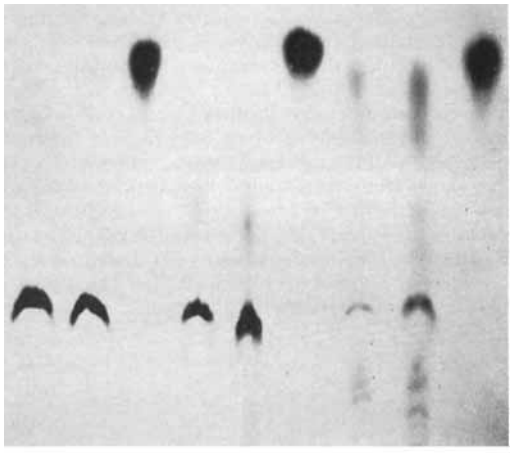

Fig. 1—Radioautograph of a paper chromatogram of urine samples applied directly without prior treatment or extraction.

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[1] Marketed as Serax by Wyeth Laboratories, Inc., Philadelphia, Pa.

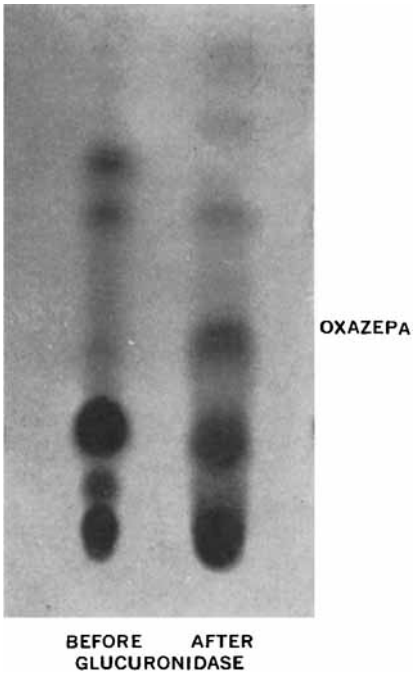

Fig. 2—Chromatogram made from urine collected between 24 and 48 hr. following a dose of ^{14}C-tagged drug to an animal on the chronic regimen.

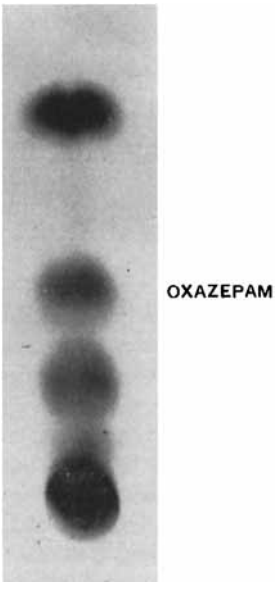

Fig. 3—Chromatogram made from feces collected between 24 and 48 hr. following a dose of ^{14}C-tagged drug to an animal on the chronic regimen.

authentic oxazepam. The chromatogram shown in Fig. 2 was made from urine collected between 24 and 48 hr. following a dose of ^{14}C-tagged drug to an animal on the chronic regimen. The oxazepam area accounts for about 16% of the radioactivity in this sample, as determined by scintillation counting. The corresponding area obtained from an extract of the 24–48 hr. feces collection (Fig. 3) of an acutely dosed animal contained 13%. The thin-layer systems which gave the best resolution of oxazepam were: (*a*) benzene–pyridine–acetic acid (80:5:2) on Whatman Silica Gel SG 41; (*b*) *n*-butanol–*n*-butyl ether–acetic acid (4:8:1) on MN-Kieselgel G-HR; (*c*) heptane–chloroform–ethanol (5:5:1) on Silica Gel G. Co-chromatography of extracts containing added nonradioactive oxazepam resulted in a single homogeneous spot at the R_f of oxazepam at which HCl-induced fluorescence corresponded exactly with the dark area on the radioautograph.

Prolonged administration of chlordiazepoxide appeared to have no effect on the extent of its conversion to oxazepam (Table I). Total amounts converted ranged from 2.4% in the acute study to 4.5% in the chronic study.

TABLE I—OXAZEPAM-^{14}C EXCRETION BY CHLORDIAZEPOXIDE-^{14}C-DOSED DOGS

	Values in % of Dose					
	Urine				Feces	
		$EtCl_2$ Extracts				$EtCl_2$ Extracts
		After β-Gluc.				
	Total ^{14}C	Before β-Gluc.	Oxazepam	Residuum	Total ^{14}C	Oxazepam	Residuum
A[a]	25.7	12.4	1.1	6.5	49.3	1.3	16.9
C[a]	23.4	10.4	1.2	6.6	61.9	3.3	42.4

[a] A, acute; C, chronic.

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