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The biotransformation of isosorbide dinitrate in dogs and humans*

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There has been interest recently in the metabolic fate of the various organic nitrate coronary vasodilators and the mechanism by which they are inactivated. Nitroglycerin has been reported to be partially metabolized to inorganic nitrite by rabbit liver homogenates.[1] Needleman and Krantz[2] have shown that nitroglycerin is metabolized to 1,2 and 1,3-dinitroglycerin, and these compounds are resistant to further degradation. They appear as urinary metabolites after the administration of nitroglycerin and are less active than the parent compound as coronary vasodilators. Pentaerythritol tetranitrate has been shown to be denitrated[3] to pentaerythritol and its mononitrate. Previous work in this laboratory† has shown that 1-chloro-2,3-propandiol dinitrate is metabolized to a mixture of the mononitrates and is excreted as their glucuronic acid conjugates. It was of interest to see if isosorbide dinitrate (ISD) is denitrated also, and if humans as well as dogs can metabolize it.

ISD was supplied as Isordil® by Ives Laboratories, Inc. Three female mongrel dogs were fasted for 24 hr and the urine was collected as a control. The dogs were allowed food for 24 hr and on the next day 100 mg of ISD was administered orally. The dogs were again fasted for 24 hr and the urine was collected for 24 hr after the administration of the compound. Nonfasted 24-hr urine specimens were taken from each of 10 patients on a regimen of from 80–160 mg ISD/day. The test urines from both dogs and humans were evaporated to a volume of about 50 ml at 40° *in vacuo*. Each 50-ml sample was then extracted three times with 150 ml of ethyl acetate and the extracts were pooled. The solvent was then dried over anhydrous sodium sulfate, evaporated to dryness at 40° *in vacuo*, and the residue taken up in 0·5 ml of ethyl acetate. A 10-μl portion of the extracts of control dog urines, of the test dog urines, and of the test human urines was spotted on chromatographic plates coated with silica gel (0·25 mm) which had been activated at 100° for 30 min. The plates were developed by ascending techniques with two solvent systems, one a benzene:ethyl acetate (1:1) mixture, and the second a 2-propanol: ammonium hydroxide (4:1) mixture. The plates from each solvent system were sprayed with a 1% diphenylamine in methanol solution and exposed to an intense source of ultraviolet light for 5 min, for the purpose of visualizing the organic nitrate esters.

Table 1 gives the R_f values of the urine samples and reference compounds in a benzene:ethyl acetate solvent system sprayed with 1% diphenylamine, and the R_f values for a 2-propanol: am-

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monium hydroxide solvent system. Two spots appeared on each plate from each solvent system when sprayed with the 1% diphenylamine reagent, but no spots appeared in the control urines and no spots were found in the test urine corresponding to that for ISD. One dog excreted 5-isosorbide mononitrate (5-ISM); one dog excreted 2-isosorbide (2-ISM) mononitrate; and the third dog excreted both at approximately the same concentration of 1 mg. Three human subjects excreted 2-ISM; five human subjects excreted 5-ISM; and no detectable metabolites were found in two human subjects.

TABLE 1. COMPARISON OF URINARY METABOLITES OF ISD TO SELECTED REFERENCE COMPOUNDS

Source of urine		R_f values* Benzene: Ethyl acetate (1:1)	2-Propanol: ammonium Hydroxide (4:1)
Control dog	A	No spots appeared	No spots appeared
	B	No spots appeared	No spots appeared
	C	No spots appeared	No spots appeared
Test dog	A	0·20	0·72
	B	0·24	0·68
	C	0·20 0·24	0·68 0·72
Test human	A	0·20	0·72
	B	0·20	0·72
	C	0·20	0·72
	D	0·24	0·68
	E	No spots appeared	No spots appeared
	F	No spots appeared	No spots appeared
	G	0·24	0·72
	H	0·24	0·68
	I	0·20	0·68
	J	0·20	0·72
ISD		0·68	0·77
2-ISM		0·24	0·68
5-ISM		0·20	0·72

* No change occurred in R_f values when a mixture of ISD, 2-ISM, and 5-ISM was chromatographed.

The absence of ISD in the test urines indicates that ISD is completely metabolized. The pathways of metabolism appear to vary in the dog, since both the 2-ISM and 5-ISM were found in the urine of this species. Likewise, the metabolism of ISD in humans appears to be a variable process. From the intensity of the spots when compared to the spot intensities produced by known concentrations of these materials, it appears that the mononitrates account for only 1 per cent of the administered dose of ISD. It is likely, therefore, that the removal of the nitrate groups is a stepwise process ending with the completely denitrated isosorbide as the major metabolite.

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