TABLE 2. LIPID SOLUBILITY VS BILIARY EXCRETION OF METHADONE AND TWO OF ITS METABOLITES

Compound	pK_a	Partition coefficient (heptane/water)*	Biliary excretion (%)†
dl-Methadone	8·62	5·0	8·8
EDDP	10·42	0·04	36·0
EMDP	5·88	13·4	0·2

* pH 7·4 phosphate buffer at 23°.

† Percentage of the dose excreted unchanged in 4 hr after a 1 mg/kg i.v. injection of the compound, average of two experiments.

Acknowledgements—We are grateful to Eli Lilly & Company, Indianapolis, for the gift of EDDP and EMDP, to Dr. Urs Schlunegger, Forensic Medicine Institute, University of Bern, for the MS determinations, and to the U.S. National Institute of Mental Health for post-doctoral support (Dr. Baselt).

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REFERENCES

1. A. H. BECKETT, J. F. TAYLOR, A. F. CASY and M. M. A. HASSAN, *J. Pharm. Pharmac.* **20,** 754 (1968).
2. A. POHLAND, H. E. BOAZ and H. R. SULLIVAN, *J. med. Chem.* **14,** 194 (1971).
3. H. R. SULLIVAN, S. L. DUE and R. E. MCMAHON, *J. Am. chem. Soc.* **94,** 4051 (1972).
4. R. C. BASELT and L. J. CASARETT, *Biochem. Pharmac.* **21,** 2705 (1972).
5. D. SHAPIRO, *J. org. Chem.* **14,** 839 (1949).
6. V. H. COHN, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. LA DU, H. G. MANDEL and E. L. WAY), p. 17. Williams & Wilkins, Baltimore (1971).
7. R. C. BASELT and L. J. CASARETT, *Clin. Pharmac. Ther.* **13,** 64 (1972).
8. E. G. C. CLARKE (Ed.), *Isolation and Identification of Drugs,* p. 803. The Pharmaceutical Press, London (1969).
9. A. J. ATKINSON, J. MACGEE, J. STRONG, D. GARTEIZ and T. E. GAFFNEY, *Biochem. Pharmac.* **19,** 2483 (1970).

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Biochemical Pharmacology, Vol. 22, pp. 3120–3122. Pergamon Press, 1973. Printed in Great Britain.

Effects of benziodarone on the biliary excretion of bromosulfophthalein and iodipamide

(*Received* 26 *March* 1973; *accepted* 9 *May* 1973)

SOME authors assume that the limiting step of the excretion of bromosulfophthalein (BSP) is the conjugation with glutathione.[1-3] This reaction is catalyzed by *S*-arylglutathione transferase and strongly inhibited by benziodarone.[4*]

Priestley found that benziodarone markedly decreases the biliary excretion of BSP in the rat.[5] This author believes that a direct action of benziodarone on the conjugation of BSP is not sufficient to explain this effect and, therefore, he suggests that this drug may modify both the conjugation and the active transport of BSP.

* Benziodarone: 2-ethyl,3-(4-hydroxy, 3 diiodobenzyl)-benzofurane.

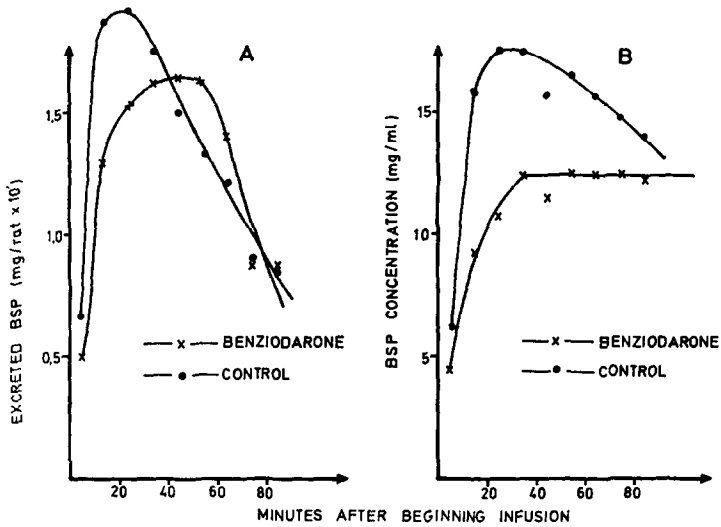

FIG. 1. BSP biliary excretion and concentration when infused into a femoral vein of a 240 g rat at a rate of 2·5 mg/min/kg for 2 hr. Benziodarone (50 mg/kg i.p.) was injected 1 hr prior to BSP infusion.

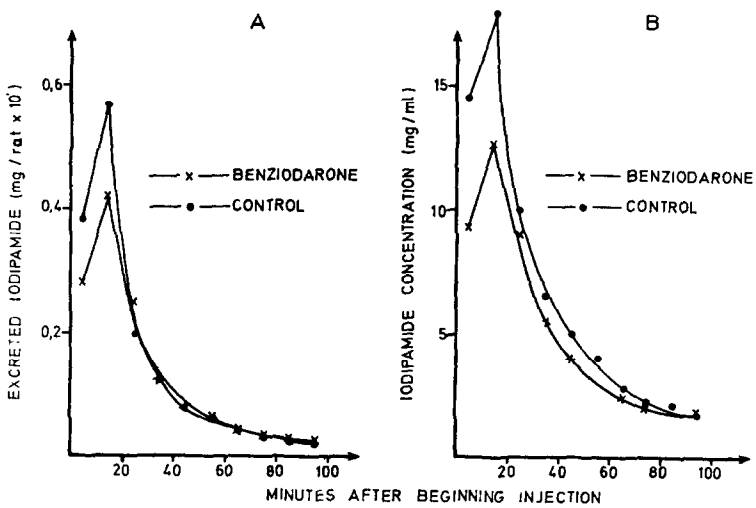

FIG. 2. Iodipamide biliary excretion and concentration when 20 μCi of sodium iodipamide —^{131}I (sp. act. 4·2 μCi/mg) were injected into a femoral vein of a 300 g rat. Benziodarone (50 mg/kg i.p.) was injected 1 hr prior to iodipamide injection.

We have confirmed Priestley's results and extended the duration of the experiments by administering a long lasting anaesthetic. We also studied the effect of benziodarone on the biliary excretion of iodipamide, a cholecystographic substance, believed to be excreted by the same mechanism.[4]

Our experiments were performed at least five times using Wistar male rats (150–350 g) from Les Oncins (France). The rats were fed a U.A.R. A04 ration (Villemoisson, France) and water *ad lib.* Inactine* (100 mg/kg i.p.), a long lasting anaesthetic, was administered 45 min after an intraperitoneal injection of benziodarone dissolved in saline (50 mg/kg). The bile duct and a femoral vein were

* Inactine = ethyl—(1 methylpropyl—thiobarbiturate sodium.

then cannulated with polyethylene tubing (PE 50). Administration of BSP or iodipamide into this femoral vein began 15 min after bile duct cannulation.

In one series of experiments, BSP was diluted with saline so that, when infused with a pump (Braun–Melsungen) at a rate of 0·033 ml/min, a dye infusion rate of 2·5 mg/min/kg was obtained. In an other series of experiments, sodium [^{131}I]iodipamide (sp. act. 4·2 μCi/mg) was injected into the venous cannula (20 mg/kg). Bile was collected for 10 min periods from the biliary cannula and biliary flow was determined gravimetrically. Biliary BSP concentrations were determined colorimetrically after dilution with 0·01 M NaOH and the absorbance was measured at 580 nm. Biliary iodipamide concentrations were obtained by counting ^{131}I radioactivity on a Phillips 4003 γ scintillation spectrometer.

Typical results, unaffected by the animal's weight, are shown in Figs. 1 and 2. Figure 1A shows that during the first 30 min of BSP infusion, the excretion of this drug was inhibited slightly in the benziodarone treated rats. This effect diminished and finally disappeared after 60 min. However, when we expressed the BSP excretion as mg/ml of bile, as Priestley has done, we found a much greater dissociation of the two curves (Fig. 1B).

A similar experiment was conducted to measure the influence of benziodarone on biliary elimination of a cholecystographic substance (iodipamide). In animals treated with benziodarone the excretion of iodipamide was inhibited slightly during the first 30 min of biliary drainage (Fig. 2A). A greater dissociation of the two curves was obtained by expressing the iodipamide excretion per ml of bile (Fig. 2B).

Thus our experiments confirm Priestley's results that benziodarone inhibits the excretion of BSP. The action of benziodarone is probably the result of an inhibition of *S*-arylglutathione transferase, since the drug inhibited also the excretion of iodipamide, which undergoes the same conjugation reaction. These inhibitions are transitory and after 60 min no significant differences can be observed.

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REFERENCES

1. J. KREBS and R. BRAUER, *Am. J. Physiol.* **37**, 194 (1958).
2. J. MELTZER, H. WHEELER and W. CRANSTON, *Proc. Soc. Exp. biol. Med.* **100**, 174 (1959).
3. G. WHELAN, J. HOCH and B. COMBES, *J. Lab. clin. Med.* **75**, 542 (1970).
4. E. BOYLAND and P. GROVER, *Clin. Chim. Acta* **16**, 205 (1967).
5. B. PRIESTLEY and G. PLAA, *Proc. Soc. exp. Biol. Med.* **132**, 881 (1969).

Biochemical Pharmacology, Vol. 22, pp. 3122–3124. Pergamon Press, 1973. Printed in Great Britain.

The metabolism of glyceryl trinitrate by liver and blood from different species*

(*Received* 28 *November* 1972; *accepted* 30 *May* 1973)

IT HAS previously been shown in rat that glyceryl trinitrate (GTN) is metabolized to the glyceryl dinitrates (GDN −1·2 and −1·3) and that these dinitrates are further metabolized to their mononitrates (GMN) and ultimately to glycerol.[1–3] Recently, Needleman *et al.*[4,5] have reported that, in rat, the major site of metabolism is in the liver, where the enzyme "organic nitrate reductase" is localized.

Crandall[6] and DiCarlo and Melgar[7] reported that GTN is enzymatically metabolized by dog blood and rat serum respectively.

In this work we have studied the *in vitro* metabolism of GTN by liver and blood from different species.

Methods. Samples for this study were obtained from rats, cats, rabbits, dogs and man. The 9000 *g* liver supernatant was prepared as described previously;[8] human liver tissue was not available. Blood

* Aided by a grant from the FWGO, Belgium.