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

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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

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samples were withdrawn from the caudal vena cava in rat, and by cardiac puncture in rabbit and cat; dog blood was taken from a vein in the foreleg and human blood was obtained from a forearm vein; 0·5 ml of heparinized undiluted whole blood was used for the incubation.

In order to determine whether the denitration takes place in the red cells or in the plasma or in both, whole blood was separated into red cells and plasma. Because of technical difficulties with hemolysis in animals, this separation was only performed with human blood. After centrifugation at 3000 rev/min for 10 min, red cells were washed three times with cold isotonic saline and resuspended with the same solution to the original volume.

The reaction medium for the liver experiments was described previously.[8] The incubation time was 10 min.

A nonenzymatic control reaction was carried out with incubation medium containing glutathione (GSH) and no tissue extract. For blood, the incubation medium was the same as that used for liver, but no GSH was added, as undiluted blood contains a sufficient amount of endogenous GSH.[9,10] The incubation time was 30 min. After incubation, GDN −1·2 and −1·3 were separated by thin-layer chromatography and a quantitative determination was made using a Vitatron Densitometer TLD 100.[11]

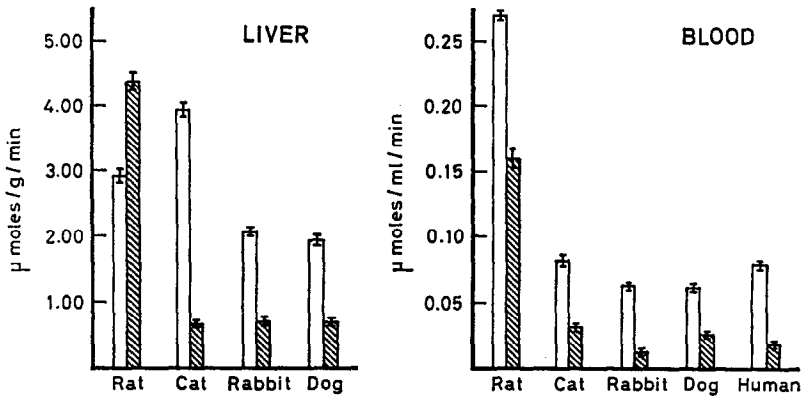

FIG. 1. Metabolism of nitroglycerin in different species. The rate of metabolism is expressed in μmoles GDN —1·2 (□) and —1·3 (▨) formed/g of liver or per ml of blood/min. The data presented are the mean values from five animals ± S.E.M. Note: different scales in liver and blood.

Results and discussion. The *in vitro* rate of GTN metabolism was determined by measurement of GDN −1·2 and −1·3 formed. No measurable amount of GMN was formed in our incubation system. The results are summarized in Fig. 1. In the nonenzymatic incubation of GTN with reaction medium containing GSH, equal quantities of both metabolites were found, as reported also by Needleman *et al.*[2] The amounts of GDN −1·2 and −1·3 formed were 0·012 ± 0·001 μmoles/ml/10 min and 0·011 ± 0·001 μmoles/ml/10 min respectively. In the enzymatic reactions, the formation of GDN −1·2 was more important than that of GDN −1·3 except in rat liver. It may be possible that during the incubation spontaneous isomerization of the 1·2 and 1·3 isomers of GDN could occur. In our previous studies, when we incubated GDN −1·2 and −1·3 separately for 30 min or 1 hr, we could not find any measurable amount of isomerization from either the GDN −1·2 or −1·3. The differences in the amount of dinitrates present are due to a different rate of formation, and not to a preferential transformation of one of the dinitrates to mononitrate. This is evidenced by the fact that no GMN could be found during the incubation either with liver or with blood. This correlates with previous findings that the enzymatic denitration of GDN is much slower than that of GTN, and that the denitration of GMN is even slower than that of the dinitrates.[2,12]

The rate of degradation of GTN is highest in rat, both in liver and blood. Cat liver appears to be more active than rabbit and dog liver in the formation of GDN −1·2. In agreement with other studies,[4,12] in rat blood more GDN −1·2 is formed than GDN −1·3, whereas in liver the reverse is true. In cat the ratio of GDN −1·2 to −1·3 is much higher in liver than in blood. In rabbit also, there is a different ratio of GDN −1·2 to −1·3 in blood and liver; in dog, there was no preferential denitration of one of the nitrate groups of the GTN molecule. The differences between liver and blood of rat, cat, and rabbit suggest the presence of isozymes of the organic nitrate reductase.

In order to study the site of denitration in blood, the rate of metabolism in human whole blood, red cells, and plasma was compared in five subjects. Whole blood metabolized GTN to GDN − 1·2 at a rate of 0·079 (± 0·003) and GDN −1·3 at a rate of 0·017 (± 0·003) μmoles/ml/min. Washed red cells showed about the same rate of denitration: GDN −1·2, 0·073 (± 0·006); GDN −1·3, 0·014 (± 0·002) μmoles/ml/min respectively. In the incubation with plasma, no metabolites could be found. Crandall[6] has reported that GTN and erythritol tetranitrate could be denitrated enzymatically by dog blood erythrocytes. However, DiCarlo and Melgar[7] reported that GTN can be metabolized by rat serum, at the temperature optimum 50–57°.

Enzymatic denitration of GTN in liver is known to be dependent on GSH.[12,13] In species so far tested where there is no plasma GSH, there is an abundance of it in the erythrocytes.[9,10] Even after addition of GSH to plasma, there was no enzymatic denitration. In contrast, in dialyzed red cells, from which the endogenous GSH has been removed, the addition of GSH restores the activity. GSH is therefore required for the metabolism of GTN in red blood cells.

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Effects of obidoxime on content and synthesis of brain acetylcholine in DFP intoxicated rats

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IN A PREVIOUS paper[1] we demonstrated that the reactivator obidoxime prevented the rise of rat brain ACh* during DFP intoxication without modification of the inhibited brain ChE activity.

Two hypotheses were suggested to explain this phenomenon: (1) the diffusion to the periphery of

*Abbreviations used: ACh, acetylcholine; ChE as used here is total capacity to hydrolyze acetylthiocholine under standard conditions; ACh synthesis refers to total ACh found in the tissue and medium after 30-min incubation period. DFP, diisopropyl phosporofluoridate; obidoxime, bis 4-hydroxyiminoethyl-pyridinium-1-methyl ether dichloride.