The faces that were to carry the hydrogens (or other substituents) were cut 0.4 mm closer to the center of the sphere than corresponding to the above-mentioned covalent radius and were provided with a socket to allow the use of standard CPK connectors. The oxygen and carbon atoms were then anodized and colored red and black, respectively. Finally, they were cemented together with epoxy glue to give the oxirane unit shown in figure 2. We are sure that the oxirane model presented here will be a valuable tool for the investigation of steric interactions either within molecules containing epoxy groups or between such molecules and a wide variety of substances.

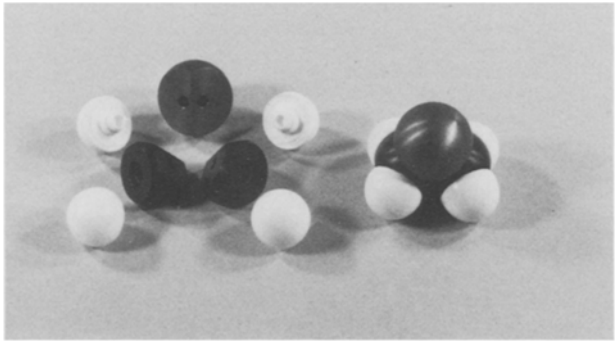

Figure 2. Oxygen and carbon atoms made from aluminium and standard plastic CPK hydrogens (left); fully assembled model of ethylene oxide (right).

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Inhibition of cytosolic rat hepatic glutathione S-transferase activities by bromosulfophthaleins

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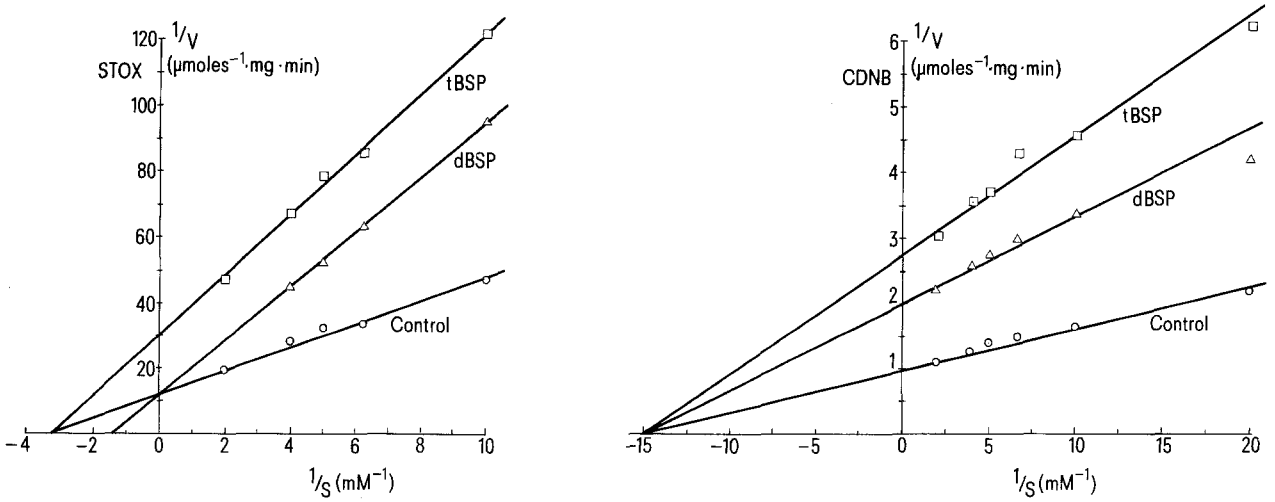
Summary. The enzymatically catalyzed conjugation of glutathione with 1-chloro-2,4-dinitrobenzene and styrene-7,8-oxide is inhibited by tetrabromosulfophthalein in a non-competetive way, although tetrabromosulfophthalein itself is a substrate for glutathione S-transferases. Dibromosulfophthalein, which is not a substrate for glutathione S-transferases, inhibits the 1-chloro-2,4-dinitrobenzene conjugation competetively and the styrene-7,8-oxide conjugation non-competetively.

Conjugation with glutathione (GSH), an important phase II reaction in the metabolism of electrophilic xenobiotics, is catalyzed by the GSH S-transferases (E.C.2.5.1.18), a family of enzymes located mainly in the cytoplasm[1]. Bromosulfophthaleins are known to interact with GSH S-transferases: 3,4,5,6-tetrabromosulfophthalein (tBSP) was shown to be a substrate[2], while 3,6-dibromosulfophthalein (dBSP) is not a substrate but was characterized as an inhibitor[3]. The bulk of enzyme activity towards tBSP and 1-chloro-2,4-dinitrobenzene (CDNB) is associated with trans-



Inhibition of rat hepatic glutathione S-transferase activities (control: ○) by dibromosulfophthalein (0.25 mM dBSP: △) and tetrabromosulfophthalein (0.05 mM tBSP: □), using 0.5 mM glutathione as the 1st substrate and styrene-7,8-oxide (STOX, left panel) or 1-chloro-2,4-dinitrobenzene (CDNB, right panel) as the electrophilic 2nd substrate, respectively.

ferase A; conjugations with epoxides such as styrene-7,8-oxide (STOX) are for the greater part catalyzed by transferase E[4].

Inhibition studies were performed with rat liver cytosolic GSH S-transferases using CDNB and STOX as substrates, and dBSP and tBSP as inhibitors. dBSP was donated by Dr D.K.F. Meijer (University of Groningen, The Netherlands), tBSP was obtained from Aldrich Chemical Co. (Milwaukee, USA). Radiolabelled STOX (7-^{3}H) with a sp. act. of 33 mCi/mmole was produced by The Radiochemical Centre (Amersham, U.K.). The enzymic conjugation of GSH with CDNB was assayed as reported before,[5] the conjugation with labelled STOX was measured according to Marniemi and Parkki[6]. Rat (♂) liver 105,000 × g supernatant served as the source of enzyme activities. In both assays the GSH concentration was fixed at 0.5 mM. The addition of inhibitor was followed by a preincubation period of 2 min after which the reaction was started by addition of the electrophilic substrate.

The figure shows experiments in which GSH S-transferase activity towards STOX or CDNB was inhibited by either dBSP or tBSP. Linear Lineweaver-Burk plots were obtained, indicating that the rate of the enzymatically catalyzed conjugations can be described by the Michaelis-Menten equation. The results show a non-competitive type of inhibition of transferase activity with CDNB as the substrate (apparent K_i of tBSP: 0.03 mM, apparent K_i of dBSP: 0.24 mM), although tBSP and CDNB are both good substrates for transferase A and show little (if any) enzymatic activity with the other transferases.[4] Obviously, the type of inhibition of dBSP is comparable with that of tBSP, albeit with an 8-fold greater apparent K_i. Surprisingly, applying STOX as the substrate results in a competetive type of inhibition with dBSP (apparent K_i: 0.19 mM) and a non-competetive type of inhibition with tBSP (apparent K_i: 0.03 mM), although tBSP has little or no activity towards transferase E, and dBSP is not a substrate at all.[4]

As, in vivo, tBSP is partly biotransformed to its GSH-conjugate, while dBSP is not metabolized[7], these compounds seem to be useful tools for the study of GSH S-transferases and the involvement of this enzyme system in the disposition of xenobiotics in vivo as well as in vitro. It should be noted however that GSH S-transferase B has been identified as the cytosolic binding protein ligandin,[1] which might be of significance with respect to the overall inhibition effects reported here.

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Reactivation of yeast glucose-6-phosphate dehydrogenase denaturated by saturated fatty acids[1]

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Summary. Activity of yeast glucose-6-phosphate dehydrogenase, inactivated by treatment with saturated fatty acids, can be partially restored by incubation in a medium of suitable ionic composition. The effectiveness of ions in the reactivation process is inversely related to their 'chaotropic' properties. Time-dependence of reactivation extent suggests a 2-step mechanism of enzyme inactivation and the existence of an intermediate form that aggregates through a 2nd-order reaction, producing irreversibly inactive enzyme.

Incubation of purified yeast glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP 1-oxidoreductase, EC 1.1.1.49) with saturated or unsaturated fatty acids causes a time-dependent inactivation of the enzyme. The effect seems to be dependent on the following experimental conditions: temperature, protein concentration, buffer, ionic strength, fatty acid concentration and chain length, presence of ligands such as NADP(H), glucose-6-phosphate and 2′-AMP[2]. In the present work some experiments are reported about the reactivation of glucose-6-phosphate dehydrogenase inactivated by myristic acid. According to the data obtained, a plausible model for the mechanism of the denaturation process promoted by fatty acids has been postulated.

Materials and methods. NADP, glucose-6-phosphate, triethanolamine · HCl were obtained from Boehringer, Mannheim, FRG. Myristic acid and other reagents were analytical grade products from E. Merck, Darmstadt, FRG. Purified *S. cerevisiae* glucose-6-phosphate dehydrogenase (grade I) was purchased from Boehringer. Before use, the enzyme was dialyzed against 100 mM triethanolamine, pH 7.6. The enzyme was NADP-free, as shown by spectrophotometric measurements of E_{278}/E_{259} ratio and E_{340} before and after addition of glucose-6-phosphate. The concentration of the apoenzyme was estimated from the absorbance at 278 nm, assuming an $\varepsilon^{1‰}_{1cm} = 0.965$[4]. The enzyme activity was measured as described by Bücher et al.[5] at 30 °C and 340 nm, with a Gilford 3400 spectrophotometer. Incubation with potassium myristate was carried out in 50 mM triethanolamine, pH 7.6 at 30 °C. The concentration of enzyme protein was 0.1 mg/ml. In the reactivation experiments, fatty acid-treated enzyme was diluted 1000-fold in a mixture containing 0.3 mg/ml of bovine serum albumin, the indicated salt and buffer.

Results. If glucose-6-phosphate dehydrogenase is inactivated by potassium myristate or by other long-chain saturated fatty acids, reactivation can occur by simple dilution in a suitable ionic environment. Figure 1 shows the recovery of activity of the enzyme pre-treated with myristate, when diluted 1000-fold in media of increasing ionic strength and