Taking into account that the two drugs, phenobarbital and SKF 525 A, have an opposite action on the drug-metabolizing enzyme activity, the assumption that these enzymes have a role in the genesis of the ethanol-induced fatty liver seems untenable.

However, an explanation of our results can be suggested. Both phenobarbital (18) and SKF 525 A[19] are known to bound to liver microsomes, thus inhibiting the activity of the enzymes which provoke the peroxidation of microsomal structural lipids.

Therefore, the protection, that we observed, may be due to a partial inhibition of the pro-oxidative effect of ethanol on the hepatic microsomes. From this point of view, the protective mechanism of the two drugs seems to be similar to that of antioxidants.

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The binding of some phenothiazines to human serum *in vitro*

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THE BINDING of drugs to serum protein can modify their tissue penetration and thus influence their concentrations at the receptor level. It has been postulated[1] that chlorpromazine is bound to serum proteins to a great extent. Nevertheless, chlorpromazine injected i.v. into rabbits rapidly disappears from the circulation, and the drug left in the blood is found mainly in the blood cells.[2] Because differences in the uptake of different phenothiazines by thrombocytes and erythrocytes have been

demontrated,[2, 3] we investigated whether these differences might correlate to varying binding of these drugs to serum proteins.

METHODS

The Sephadex batch method[4] was applied for the measurement of the protein binding of phenothiazines. This allows the unbound drug to enter the internal volume of Sephadex through pores, while large protein molecules with bound drug are excluded. The drug then is taken from Sephadex into water or saline.

Fifty mg of Sephadex (G-25 coarse particle size 100–300 μm, Pharmacia, Uppsala) was placed in a glass tube with a nylon mesh bottom, and allowed to swell for 60 min in saline solution to minimize the protein absorption on it.[5] The Sephadex gel then was left in contact with 1·0 ml of saline or serum containing known amounts of phenothiazines. After 30 min the Sephadex tube was removed and its excess moisture dried in slight vacuum. Immediately after that the tube was put into a small crucible containing 1·4 ml of 0·1 N hydrochloric acid for 15 min where it was moved up and down gently at 5 min intervals. The experiments were done at room temperature (23°) as we found that the results were about the same as at 37°.

The phenothiazines used were chlorpromazine hydrochloride (May & Baker Ltd., Dagenham), desmonomethylchlorpromazine maleate, chlorpromazine sulphoxide (Rhône-Poulenc, Paris), and a quaternary compound *N*-hydroxyethylpromethazine hydrochloride (Orion Oy, Helsinki). Their concentrations in the eluate and parent solution were estimated spectrophotometrically as described by Ahtee.[3]

RESULTS

As can be seen in Table 1, the uptake of chlorpromazine, desmonomethylchlorpromazine, and *N*-hydroxyethylpromethazine by Sephadex from saline was clearly higher than from serum in every studied concentration. Chlorpromazine sulphoxide was taken up from saline approximately like the other drugs studied, but its uptake by Sephadex from serum was only slightly lower than from saline. Since 10-fold concentrations of different phenothiazines with different p*Ka* values gave about similar uptakes from saline, the degree of ionization does not modify their diffusion to Sephadex. The recoveries of chlorpromazine sulphoxide from serum and from saline were about similar. This finding provides an indirect evidence for about similar distribution coefficients for drugs between serum and Sephadex or saline and Sephadex. Therefore, the reduced recovery of the other drugs from serum is plausibly due to their binding to serum proteins. Since the total amounts of drugs measured in saline or serum were similar, it excludes their loss into Sephadex as a possible cause for the reduced recovery from serum.

The reduced uptake of chlorpromazine from serum corresponds to the protein binding of about 60 per cent. The uptake of desmonomethylchlorpromazine from serum indicates it is bound to serum in a corresponding degree. On the other hand, the similar uptake of chlorpromazine sulphoxide both from saline and from serum indicates a low degree of protein binding, approximately 20 per cent. The uptake of the quaternary *N*-hydroxyethylpromethazine was slightly higher than that of chlorpromazine, indicating a 50 per cent protein binding.

DISCUSSION

The data presented confirm the early finding of Salzman and Brodie[1] of the considerable protein binding of chlorpromazine. Its practical meaning is not clear, because the character of the protein binding of drugs[6] is at least as important as its quantity. The present results explain, at least partly, why phenothiazines exhibit higher haemolytic activity *in vitro* when using saline-red cell suspensions than when using the whole blood.[3]

Chlorpromazine and its desmonomethyl derivative behaved in our experiments in the same way. These compounds accumulate in the red cells in similar amounts,[3] while the accumulation of desmonomethylchlorpromazine in the thrombocytes is superior to that of chlorpromazine.[3] On the basis of the present results, the latter effect is not due to different concentrations of diffusible drugs in plasma.

The serum protein binding of chlorpromazine sulphoxide, the accumulation of which in red cells is approximately half of that of chlorpromazine,[2, 3] was the least of the drugs studied. We recently

TABLE 1. THE UPTAKE BY SEPHADEX GEL OF SOME PHENOTHIAZINE DERIVATIVES FROM SALINE OR HUMAN SERUM AT 23°
Drug eluted refer to the amount of drug diffused to Sephadex. Recovery % refer to the relation drug eluted:total drug.

Drugs M	Uptake of drugs from saline			Uptake of drugs from serum		
	Drug eluted (μg/ml)	Drug left in parent solution (μg/ml)	Recovery (%)	Drug eluted (μg/ml)	Drug left in (μg/ml)	Recovery (%)
Chlorpromazine						
3×10^{-4}	8·4 ± 2·0	37·9 ± 4·8	18	2·7 ± 0·4	60·9 ± 4·1	4
10^{-3}	24·3 ± 4·5	161·8 ± 9·7	13	9·8 ± 1·4	200·5 ± 7·3	5
3×10^{-3}	78·2 ± 12·2	542·2 ± 35·3	13	33·5 ± 2·7	651·6 ± 20·8	5
At 37° 10^{-3}	36·0 ± 3·4	187·7 ± 4·6	16	11·0 ± 0·4	244·7 ± 4·0	4
Desmonomethylchlorpromazine						
3×10^{-4}	6·1 ± 1·2	62·7 ± 5·3	9	2·4 ± 0·3	63·9 ± 5·7	4
10^{-3}	20·6 ± 1·7	187·4 ± 3·1	10	7·7 ± 0·8	205·3 ± 11·2	4
3×10^{-3}	57·0 ± 8·0	543·0 ± 59·2	10	23·8 ± 2·6	606·4 ± 19·0	4
Chlorpromazine sulphoxide						
3×10^{-4}	11·8 ± 0·6	83·3 ± 2·8	12	7·6 ± 1·1	80·4 ± 3·2	9
10^{-3}	30·4 ± 2·2	266·7 ± 14·1	10	22·5 ± 1·7	277·1 ± 17·2	8
3×10^{-3}	8·51 ± 7·6	810·1 ± 36·0	10	65·9 ± 5·8	862·9 ± 47·2	7
N-Hydroxyethylpromethazine						
3×10^{-4}	7·7 ± 0·8	49·9 ± 5·7	13	4·5 ± 0·5	57·4 ± 7·0	7
10^{-3}	23·8 ± 2·2	140·5 ± 8·8	14	11·2 ± 0·8	151·0 ± 10·2	7
3×10^{-3}	77·6 ± 3·9	474·3 ± 30·1	14	37·1 ± 3·5	498·6 ± 29·2	7

found a comparable phenomenon in the subcellular distribution of phenothiazines in the rabbit platelets, where the main part of chlorpromazine sulphoxide remained in the supernatant while chlorpromazine and desmonomethylchlorpromazine were bound to particulate fractions.[7] Both these phenomena correlate to the low lipid-solubility of chlorpromazine sulphoxide.[7]

The quaternary *N*-hydroxyethylpromethazine, being poorly lipid-soluble,[7] is taken up by red cells even less than chlorpromazine sulphoxide[2, 3] but its protein binding was relatively high. Therefore, the protein binding of the drugs cannot be solely correlated to their lipid-solubility but other physicochemical properties of the drugs are also of importance.[8]

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Blood concentrations of *N,N'*-trimethylenebis(pyridinium-4-aldoxime) (TMB-4) and *N,N'*-oxydimethlenebis (pyridinium-4-aldoxime) (toxogonin) after intravenous and intramuscular administration in the dog

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BLOOD concentrations of *N,N'*-trimethylenebis (pyridinium-4-aldoxime) (TMB-4) and *N,N'*-oxydimethylenebis (pyridinium-4-aldoxime) (Toxogonin, Lü H6) at various times after intravenous and intramuscular administration of oximes (20 mg/kg) have been estimated in dogs anaesthetized with chloralose. The half-life was 28·3 min for TMB-4 and 19·9 min for Toxogonin after i.v. injection. The rates of absorption of either oxime were equal and the maximum blood concentrations were reached within 15 min after intramuscular injection.

In a series of papers,[1, 2, 3] Erdmann and coworkers have tried to show that Toxogonin is in many respects superior to pralidoxime (2-PAM) and TMB-4 as an antidote against organophosphorus anticholinesterases. However, Heilbronn and Tolagen[4] have found that the cholinesterase reactivating power of Toxogonin in experimental sarin or tabun poisoning is comparable to that of TMB-4. Hobbiger and Vojvodić,[5] also, could not find any substantial difference in reactivating and antidotal effects of TMB-4 and Toxogonin in mice and rats poisoned with several organophosphorus compounds.