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PROTECTION OF RATS AGAINST THE HEPATOTOXIC EFFECT OF PARACETAMOL

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Summary.—In view of increasing knowledge of the mechanism of production of hepatic damage by paracetamol, and the results of recent studies suggesting a beneficial effect from cysteamine administered soon after an overdose, studies were carried out in the rat on a number of possibly protective agents, using oral paracetamol in a dose of 2·5 g/kg body weight. Histological evidence of liver damage was reproducibly obtained with corresponding reductions in the levels of cytochrome P450. This was completely prevented by prior oral administration of cysteamine in a dose of 300 mg/kg body weight. The levels of cytochrome P450 were also maintained following an intraperitoneal injection of α-tocopherol (400 mg/kg body weight) but the effect on the histological evidence of liver damage was less. The administration of glutathione, propranolol and thioctic acid did not prevent the liver damage, although with these agents—except glutathione—the number of wedge shaped areas of necrosis (infarcts) in the liver was reduced.

HEPATIC damage and death following a paracetamol overdose were first reported from Britain in 1966 (Davidson and Eastham) and since that time there has been a rapid increase in the use of this drug in self poisoning (Clark *et al.*, 1973). The mechanisms by which paracetamol produces liver damage have recently been elucidated (Mitchell *et al.*, 1973*a*, *b*; Jollow *et al.*, 1973; Potter *et al.*, 1973), on the basis of which it may be possible to develop a more effective treatment for patients suffering from an overdose (Prescott *et al.*, 1974). Paracetamol is thought to be metabolized in the liver to a reactive intermediate by a mixed function oxidase enzyme dependent upon cytochrome P450. Induction of this enzyme system by the administration of phenobarbitone enhances the degree of liver damage. The reactive intermediate is normally conjugated with glutathione but when the hepatic stores of this are reduced, it may combine with and damage tissue protein. Thus, the mechanism of production of liver damage by paracetamol may be similar to that described for irradiation injury when active free radicals are formed. A number of agents have been shown to protect experimental animals from damage caused by irradiation and in this paper we report a study of their effectiveness on the degree of liver necrosis produced in rats by paracetamol.

MATERIALS AND METHODS

A strain of male albino Wistar rats maintained in the animal house of King's College Hospital Medical School was used, the animals being fed on a standard laboratory diet

(Oxoid modified MRC diet 41B) with drinking water freely available. Nine groups, comprising 8 rats in each, were studied. With the exception of the animals in the first control group (A), all rats received phenobarbitone in their drinking water (1 g/l) for 72 hours. Group B constituted phenobarbitone treated (enzyme induced) controls, Groups C–E being given an aqueous suspension of paracetamol 2·5 g/kg body weight (Winthrop Ltd), *via* an intragastric tube following the induction period. The protective agents under study were given immediately before administration of paracetamol, Group D receiving intravenous glutathione (40 mg/kg body weight, Laboratorie Jouliée), Group E propranolol (40 mg/kg body weight, Imperial Chemical Industries) and Group F thioctic acid (5 mg/kg body weight, Richardson-Merrell). These groups also received a further dose of the protective agent 4 h after the first. Group G received cysteamine (300 mg/kg body weight, Sigma) as a single oral dose, Group H the same dose *via* an intraperitoneal injection and the final Group I were given α-tocopherol acetate (400 mg/kg body weight, Sigma) by intraperitoneal injection.

The animals were sacrificed 48 h after the induction period and the livers rapidly removed. Portions (3 g) were placed in ice and used for cytochrome P450 estimations, the remainder being fixed in formol saline.

Histological assessment.—Sections taken from 3 lobes of each liver were examined histologically by one of us (B.P.) without knowledge of the animal group. Necrosis was graded as follows: None (Grade 0), restricted to the centre of the lobule (Grade 1), centrilobular necrosis with extension to the portal tracts (Grade 2), and more extensive necrosis throughout the lobule with a thin rim of normal cells around the portal tract (Grade 3). An assessment of the degree of overall liver damage was obtained from the numerical average of the histological grading for each of the 3 lobes. Wedge shaped areas of confluent necrosis often surrounded by normal liver tissues were noted separately.

Cytochrome P450.—The levels of cytochrome P450 were determined in samples of liver from 4 rats in each group by the method of Omura and Sato (1964) and related to microsomal protein (Lowry *et al.*, 1951).

RESULTS

The extent of necrosis varied little between the 3 lobes of liver examined in each animal, and the variation between the animals in each group was also acceptable, as shown in Table 1. Of the protective agents, only oral cysteamine com-

TABLE I.—*Grading of Liver Damage on Histological Examination found in the Different Groups of Animals*

Group	Histological assessment: Individual grade	Histological assessment: Mean for group	Wedge shaped necrosis (No. of rats)
C (Phenobarbitone, paracetamol)	32322223	2	5
D (Phenobarbitone, paracetamol, glutathione)	23223232	2	6
E (Phenobarbitone, paracetamol, propranolol)	23111332	2	2
F (Phenobarbitone, paracetamol, thioctic acid)	13122232	2	2
G (Phenobarbitone, paracetamol, cysteamine oral)	00000000	0	0
H (Phenobarbitone, paracetamol, cysteamine i.p.)	1110 Died	1	0
I (Phenobarbitone, paracetamol, α-tocopherol)	22132222	2	1

pletely prevented necrosis. When cysteamine was given intraperitoneally the protection was less and half these animals died with convulsions within 24 h of administration, the reasons for which were not apparent at autopsy. No animals died within the 48 h period in the other groups. The effect of the other agents on the grading of histological liver damage was less marked although all of them with the exception of glutathione, reduced the numbers of wedge shaped areas of confluent necrosis.

Changes in cytochrome P450

The levels in the control rats given phenobarbitone alone (Group B) were significantly greater ($P < 0.01$) than in the non-induced controls (Group A, Table II). In the Group C animals given paracetamol after the period of induction,

TABLE II.—*Results of Cytochrome P450 Levels. Mean Values for 4 Animals from each Group Together with the Standard Error are Given*

Group of animals	Cytochrome P450 (nmol/mg microsomal protein)	Statistical difference of treatment group from Group C (P)
A (Controls)	0·65±0·01	
B (Phenobarbitone)	0·82±0·04	
C (Phenobarbitone, paracetamol)	0·49±0·03	
D (Phenobarbitone, paracetamol, glutathione)	0·56±0·13	>0·05
E (Phenobarbitone, paracetamol, propranolol)	0·33±0·06	>0·05
F (Phenobarbitone, paracetamol, thioctic acid)	0·69±0·08	>0·05
G (Phenobarbitone, paracetamol, cysteamine oral)	0·98±0·10	<0·005
I (Phenobarbitone, paracetamol, α-tocopherol)	0·79±0·09	<0·05

enzyme levels were significantly lower than in either Group A ($P < 0.01$) or Group B ($P < 0.001$). Of the animals receiving protective agents, only those given oral cysteamine and α-tocopherol had significantly higher levels of cytochrome P450. Indeed, in these 2 groups the levels of cytochrome P450 were as high as in Group B—the animals given phenobarbitone alone without paracetamol.

DISCUSSION

Oral cysteamine completely prevented liver damage when given at the same time as a large dose of paracetamol, without any apparent side-effects. The same dosage given intraperitoneally provided less effective protection and half the animals died. It is possible that the oral dose was more effective and less toxic because of slow absorption over a number of hours. Cysteamine may act by combining with, or preventing the formation of the reactive intermediate, or else by increasing the glutathione stores in the liver. Levels of glutathione have been shown to increase when hepatic microsomal enzymes are incubated with cysteamine *in vitro* (Révész and Modig, 1965).

The protective effects of cysteamine (in a smaller dose of 40 mg/kg body weight) in man have recently been reported by Prescott and his colleagues (1974). The drug was administered intravenously to 7 patients at intervals of up to 10 h following an overdose and hepatic damage was almost completely prevented. The minimum effective dosage and the maximum time period following ingestion at which it will exert a protective effect still remain to be determined. In the patient studies referred to there were signs of gastrointestinal and central nervous system toxicity and the use of cysteamine should perhaps be confined at present to those who are most liable to develop severe hepatic lesions.

It is surprising that glutathione did not have a protective effect but it is possible that the compound was rapidly broken down in the circulation before reaching the liver. Alpha-tocopherol is an important natural antioxidant (Green *et al.*, 1967). It stabilizes microsomal membranes from attack by free radicals (McCay *et al.*, 1971) and it has recently been reported to protect rats from the effects of paracetamol (Kelleher *et al.*, 1974). In the present series of experiments,

in which smaller doses were given, the fall in levels of cytochrome P450 following paracetamol administration was prevented although it had no effect on the extent of necrosis. Propranolol has also been reported to reduce paracetamol induced liver necrosis in the mouse, perhaps by causing vasodilation, as the hepatic damage may in part be caused by vasoconstriction and secondary anoxia (Rosner, Romero-Ferret and Mottot, 1973). Indeed, the wedge shaped areas of liver necrosis which we found in all groups of rats receiving paracetamol were probably the result of infarction. Propranolol as well as the other protective agents administered (except glutathione) appeared to reduce the number of these segmental lesions. Thioctic acid which has been used in the treatment of *Amanita phalloides* poisoning, is thought to protect sulphydryl groups, which may be important for the action of reduced glutathione and cysteamine, but it did not reduce the degree of paracetamol induced liver necrosis in our experiments. Why some of these protective agents reduced the degree of hepatic damage and others appeared ineffective is not clear and may be explained only when the mode of action of the reactive metabolite of paracetamol is more completely understood.

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