Effect of Anesthetic Drugs on Lysosomal Enzymes in the Rat[1] (35795)

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The choice of anesthesia for liver transplantation or for surgery on any patient having severe liver disease is difficult because many anesthetic drugs are toxic to the liver (1). Anesthetic agents with a halogenated hydrocarbon structure, which have been used without untoward effects in the management of patients undergoing hepatic transplantation, may be inplicated in parenchymal injury of the liver (2–4).

Many difficulties persist in evaluating the type, degree, and potential reversibility of liver injury produced by anesthetics. Some recent studies examined lysosomal enzymes as an index for early detection of cell injury in the liver (5–7). The stability of the hepatic lysosome has been interpreted as an indication of cellular integrity in this organ.

Carbon tetrachloride administration has been reported to produce rapid development of liver injury in the rat (8, 9). This study presents a comparison of the effects of CCl_4, with those of chloroform, and of three other halogenated anesthetic compounds [halothane (Fluothane), fluroxene (Fluoromar) and methoxyfluorane (Penthrane)] on the concentrations of two lysosomal enzymes in the liver and plasma of rats.

Materials and Methods. Five groups of four to six Sprague-Dawley male rats (90 to 100 g in wt, in the fasting state) were exposed to various volatile anesthetic agents (Table I). The anesthetics, mixed with air and oxygen enough to attain 25% oxygen, were vaporized with a total flow of 5 liters/min into a chamber previously described (10); each group of the rats was anesthetized, demonstrated by disappearance of the righting reflex, for 30 min. The concentrations of the vaporized agents were measured in a gas chromatograph (11) and are shown in Table I. At the end of this procedure, liver biopsies and blood samples were immediately obtained for enzyme determinations while the rats were still under anesthesia. One more group used as control was placed in the chamber for the same period with a mixture of air and oxygen; specimens were obtained immediately after decerebration.

The liver specimens were homogenized in cold 0.25 *M* sucrose with Dounce homogenizers. Plasma was obtained from the blood by centrifugation in the cold for 10 min at 1500 rpm. The activity of acid phosphatase in the liver homogenates and in the plasma was determined by the method of Lazarus and Scherbaum (12); *beta*-glucuronidase was determined by the method of Gianetto and De Duve (13). The incubation conditions were 60 min at 37° for *beta*-glucuronidase and 60 min at 29° for acid phosphatase (acetate buffer, pH 5.0).

Results. The levels of the two lysosomal enzymes in the liver after the various treatments are presented in Table I. All of the treatments resulted in a significant increase

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TABLE I. Enzyme Activities (units of enzymes/g of wet wt/min) in Homogenates of Rat Liver Following Inhalation of Various Anesthetic Drugs.
The drug concentrations were constant for the 30 min of exposure.

Anesthetic agent	Drug conc (%; v/v)	Acid phosphatase M	Acid phosphatase SD	*Beta*-Glucuronidase M	*Beta*-Glucuronidase SD
Control (air) (6)[a]	—	9.27 ± 0.38		0.185 ± 0.029	
Carbon tetrachloride (6)	1.2	9.91 ± 3.73		0.297 ± 0.076[c]	
Chloroform (4)	0.8	13.50 ± 4.57[c]		0.640 ± 0.075[c]	
Methoxyfluorane(6)	0.24	18.29 ± 2.38[c]		0.298 ± 0.043[c]	
Halothane (5)	1.1	13.72 ± 1.44[c]		0.358 ± 0.043[c]	
Fluroxene (6)	5.1	11.76 ± 1.95[b]		0.393 ± 0.122[c]	

[a] Number of rats in each group.
[b] When compared with controls: $p < 0.05$; [c] $p < 0.01$.

in *beta*-glucuronidase and all drug treatments except carbon tetrachloride resulted in increased acid phosphatase activity. The activity of acid phosphatase in methoxyfluorane-treated rat livers was higher than in any of the other groups ($p<0.01$). The acid phosphatase levels for chloroform and halothane-treated rats were significantly higher than in controls or in rats treated with fluroxene or carbon tetrachloride ($p<0.01$). A completely different pattern was observed in the *beta*-glucuronidase levels; the level was higher for animals treated with chloroform than for all of the other groups ($p<0.01$).

The levels of the enzymes in plasma differed considerably from the liver levels (Table II). All of the treatments resulted in increased plasma acid phosphatase. The plasma levels of acid phosphatase in the animals treated with carbon tetrachloride or methoxyfluorane were significantly higher than in the other groups ($p<0.01$). An increase in plasma *beta*-glucuronidase was associated with all treatments except halothane. Carbon tetrachloride treatment resulted in a higher level of *beta*-glucuronidase than did treatment with the anesthetic drugs ($p<0.01$).

Discussion. Carbon tetrachloride has been shown in other studies to be toxic to the liver (8, 9, 14) although the method of administration in those studies was by oral intubation rather than by inhalation. In the present investigation, the marked increase in plasma lysosomal enzymes following treatment with carbon tetrachloride probably reflects damage to some part of the body, including the liver. The lack of marked increase in liver lysosomal enzymes may reflect a "leakage" of enzymes from the liver into the blood, as suggested by others (8). It is also possible that the increase in plasma enzymes may occur from a source other than the liver.

Treatment with all of the other drugs resulted in increase of lysosomal enzyme levels

TABLE II. Enzyme Activities (units of enzymes/ml/min) in Plasma of Rats Following Inhalation of Various Anesthetic Drugs.

Anesthetic agent	Acid phosphatase M	Acid phosphatase SD	*Beta*-Glucuronidase M	*Beta*-Glucuronidase SD
Control (air)	0.69 ± 0.12		0.013 ± 0.003	
Carbon tetrachloride	2.15 ± 0.34[b]		0.104 ± 0.039[b]	
Chloroform	1.44 ± 0.28[b]		0.038 ± 0.013[a]	
Methoxyfluorane	1.86 ± 0.12[b]		0.069 ± 0.017[b]	
Halothane	0.98 ± 0.08[a]		0.015 ± 0.005	
Fluroxene	1.28 ± 0.26[b]		0.039 ± 0.007[a]	

[a] When compared with controls: $p < 0.05$; [b] $p < 0.01$.

in the liver and in various degrees of increase of these enzymes in the plasma (Tables I and II). If one considers the plasma concentrations as an indication of liver damage, halothane would appear to be the least toxic drug. If one considers the liver concentrations, fluroxene could be the least toxic although the liver levels of both enzymes after this treatment exceeded those observed after administering carbon tetrachloride.

Halothane has been shown to be relatively nontoxic to the liver in animal experimentation (2, 15) although severe hepatic necrosis has been reported in clinical situations (16, 17) after repeated exposure. Fluroxene has been thought to be a relatively safe agent since no modifications of liver function tests have heretofore been observed (1).

It appears that the determination of lysosomal enzyme levels may be a promising means of appraising the response of an organ to injury. Considerable work remains to be done in the evaluation of the potential relationships between observed changes in enzyme levels within an organ and concomitant changes in enzyme levels in the circulation.

Summary. A comparison of the effects of carbon tetrachloride with those of chloroform and of three other halogenated anesthetic compounds on the concentrations of two lysosomal enzymes in the liver and plasma of rats is presented. In the liver, all of the treatments resulted in an increase in *beta*-glucuronidase and all except carbon tetrachloride resulted in increased acid phosphatase activity. The plasma levels of acid phosphatase increased after all treatments while an increase in plasma *beta*-glucuronidase was associated with all treatments except halothane. The degree of increased acid hydrolase levels varied between the treatments both in the liver and in the plasma.

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