Effects of Phenobarbital Pretreatment on the Response of Rat Liver to Halothane Administration[1] (36666)

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In man, anesthesia with 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane) is followed on rare occasions by the development of massive hepatic necrosis (1–4). The reasons for this untoward development are by no means clear, although there is some evidence that hypersensitivity has a role (3, 5–9). Thus far, in experimental animals, such catastrophic effects upon the liver have not been produced, even by repeated or sustained exposure to the anesthetic (10–13). In general, hepatic necrosis has not been a feature of halothane toxicity in the experimental animal (10–13); however, more subtle changes, perhaps indicative of liver injury, have been observed in both man and experimental animals. Thus, halothane administration has been associated with abnormal hepatic lipid accumulation (11–15), structural and functional alterations of liver mitochondria (3, 15, 16), reduced bromsulphthalein clearance (8, 17), elevations of certain plasma enzymes (8, 18), and depression of hepatocellular enzyme systems (19).

Halothane is metabolized, in part, by the microsomal drug metabolizing enzyme systems of the liver (20–23), and induction of these hepatic enzyme systems increases this metabolism (24). Since the metabolites of halothane may be toxic, it has been suggested that hepatic enzyme induction might be expected to potentiate the anesthetic's hepatotoxicity (25–27). Pretreatment with phenobarbital, a well known enzyme inducer, has already been shown to enhance the hepatotoxicity of another halogenated hydrocarbon, *viz.*, carbon tetrachloride (28–30). The present investigation, therefore, was initiated to determine whether such enzyme induction would affect the response of rat liver to halothane administration.

Materials and Methods. Male albino (Holtzman) rats, weighing 60–80 g, were injected ip on each of 3 successive days with phenobarbital (75 mg/kg) in an isotonic saline solution. Control animals received only saline. All rats were fasted for 18 hr; and then half of the phenobarbital and half of the saline pretreated rats were anesthetized by ip injection of 0.5 ml of a 25% solution of halothane in olive oil. This dose maintained anesthesia for approximately 2 hr. For anesthetic intervals of 4 or 8 hr, additional 0.25–0.5 ml doses of the halothane solution were given, as required. The remaining phenobarbital and saline pretreated animals, serving as anesthetic controls, were injected ip with equivalent amounts of pure olive oil.

Cytochrome P-450 levels were determined for hepatic microsomal suspensions prepared from rats in each of the four categories of treatment. After 2 or 4 hr of anesthesia, the microsomes were analyzed immediately. After 8 hr of anesthesia, the microsome were frozen overnight and analyzed the following day. Livers from 3 rats in each category were pooled for each of 3 separate determinations at each anesthetic interval. The methods of microsomal preparation and analysis were similar to those of Omura and Sato (31) with minor modifications, as previously reported (32). Protein determinations were made according to the method of Lowry *et al.* (33), and microsomal suspensions were then di-

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luted to a uniform concentration of 2 mg protein/ml. Aliquots were delivered into cuvettes of 1 cm light path and reduced by addition of 10 mg of sodium hydrosulfite (dithionite). Carbon monoxide was bubbled through the microsomal suspension in the sample cuvette for 1 min. Then both sample and reference cuvettes were capped with Teflon and the difference in absorbance at 450 and 490 nm (Δ OD 450–490 nm) was determined in a Beckman dual-beam spectrophotometer.

Paralysis times were measured for ten rats in each of the treatment categories, at 1 hr after the conclusion of a 4-hr anesthetic interval. Paralysis was induced by zoxazolamine, prepared by the method of Conney *et al.* (34), and administered ip in a dose of 100 mg/kg. In each instance, the paralysis time was measured from the point of complete immobilization to the point at which the animal moved to a different location in the cage.

For histologic study, animals in each treatment category were killed at 24 hr after the conclusion of anesthetic intervals of 2, 4 or 8 hr duration. There were 20 rats in each category at each interval. Sections of their livers were fixed in cold (4°) 10% formalin, buffered to pH 7.0. Paraffin sections were stained with hematoxylin and eosin for evaluation by light microscopy.

Results. The halothane solution, given in these experiments, consistently produce anesthesia. A single injection was usually adequate for a 2-hr period. For the longer anesthetic intervals, multiple doses were required; however, the total dose, even for an 8-hr interval, did not exceed 2.0 ml of the 25% solution of halothane in olive oil. Saline pretreated rats required neither more nor less of the halothane solution than the phenobarbital-pretreated animals, nor was there any apparent difference in the depth of anesthesia between these two groups.

Administration of halothane to phenobarbital-pretreated rats significantly decreased the level of hepatic microsomal cytochrome P-450 from that of the phenobarbital–olive oil-treated controls (Table I). After 2–4 hr of anesthesia, there was a 35–37% decrease, whereas 8 hr of anesthesia caused a 43% reduction from the control level. In contrast, equivalent intervals of halothane anesthesia caused no significant depression of cytochrome P-450 in the saline pretreated rats, as compared to their saline–olive oil-treated controls (Table I).

Furthermore, in phenobarbital pretreated rats, halothane anesthesia of 4 hr duration caused a significant prolongation of zoxazolamine-induced paralysis times, in comparison with those of the phenobarbital–olive oil-

TABLE I. Effects of Treatments on Hepatic Microsomal Cytochrome P-450.[a]

Treatment[b]	Anesthetic interval (hr)		
	2	4	8
Phenobarbital + halothane	0.235 ± 0.012[c]	0.255 ± 0.005[c]	0.210 ± 0.013[c]
Phenobarbital + olive oil	0.373 ± 0.014	0.393 ± 0.007	0.368 ± 0.013
Saline + halothane	0.125 ± 0.014[d]	0.129 ± 0.010[d]	0.092 ± 0.007[d]
Saline + olive oil	0.127 ± 0.013	0.126 ± 0.005	0.095 ± 0.005

[a] Values are recorded as mean Δ OD 450–490 nm ± SE. Livers from three rats were pooled for each of three separate determinations in each treatment category at each anesthetic interval.

[b] Phenobarbital (75 mg/kg) was given ip on each of 3 successive days, while control rats received only saline. On the fourth day, halothane (0.5 ml of a 25% solution in olive oil) was given ip to one-half of each group. This induced anesthesia for approximately 2 hr. For the longer periods, anesthesia was maintained by additional injections, as required. Anesthetic controls received equivalent amounts of pure olive oil ip.

[c] Significantly lower than the phenobarbital–olive oil control level by the Student's t test ($p < 0.005$).

[d] *Not* significantly different from the saline–olive oil control level by the Student's t test.

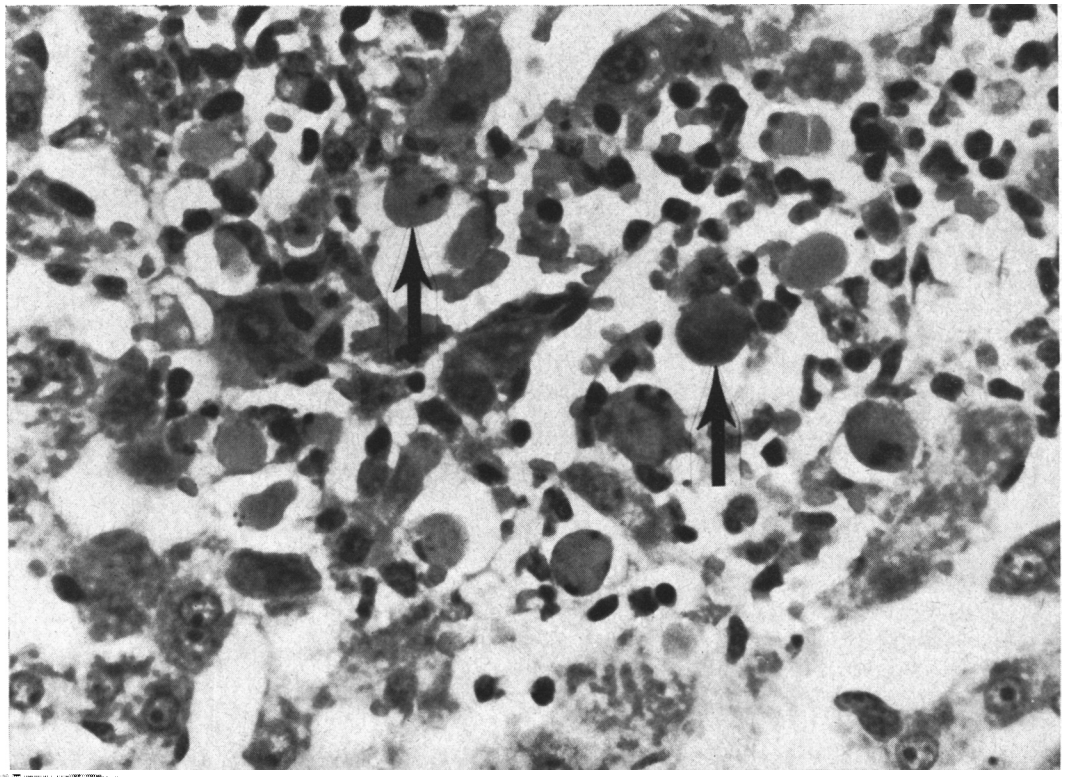

FIG. 1. Light micrograph illustrating a small focus of necrosis in the liver. This rat was pretreated with phenobarbital for 3 successive days, anesthetized with halothane for 4 consecutive hr on Day 4 and killed on Day 5 to obtain histologic sections of the liver. The discrete focus contains many dead and dying hepatocytes, which appear as shrunken, condensed, acidophilic bodies (as at arrows). Many of these cells are devoid of nuclei; others show advanced pyknosis and karyorrhexis. Mononuclear cells in moderate numbers have infiltrated the necrotic focus. Viable hepatic parenchymal cells are evident at the periphery of the field. ×570.

treated animals (Table II); whereas saline-pretreated rats, whether anesthetized or not, displayed nearly identical paralysis times (Table II).

The histologic studies revealed that halothane administration to phenobarbital-pretreated rats *consistently* produced multifocal necrosis (Fig. 1) in the liver parenchyma. Patchy subcapular necrosis (Fig. 2) was also frequently observed. The small foci of parenchymal necrosis were more prevalent after 4 or 8 hr of anesthesia than after 2 hr, and the subcapular patches were more extensive after the longer anesthetic intervals. Neither type of necrosis, however, was identified in the saline pretreated animals, whether they were anesthetized for 2, 4, or even 8 hr; nor were morphologic abnormalities identified among any of the olive-oil-treated anesthetic controls.

Discussion. The foregoing results provide evidence that, in phenobarbital-pretreated rats, administration of halothane ip can produce functional impairment of the liver. At each of the anesthetic intervals tested, cytochrome P-450, the oxygen activating component of the hepatic microsomal drug metabolizing enzyme systems, was substantially reduced from control levels. This change may be taken as an index of hepatic injury (35). Moreover, this *in vitro* evidence of injury was supplemented by the *in vivo* data, which indicated that halothane anesthesia of 4 hr duration significantly lengthened zoxazolamine-induced paralysis times in phenobarbital pretreated rats. Though not the only possibil-

TABLE II. Effects of Treatments on Zoxazolamine Paralysis Time.

Treatment[a]	No. of rats	Paralysis time (minutes)[b]
Phenobarbital + halothane	10	48.9 ± 2.9[c]
Phenobarbital + olive oil	10	36.9 ± 3.4
Saline + halothane	10	97.1 ± 10.7[d]
Saline + olive oil	10	97.0 ± 4.5

[a] Phenobarbital (75 mg/kg) was given ip on each of 3 successive days, while control rats received only saline. On the fourth day, halothane (0.5 ml of a 25% solution in olive oil) was given ip to induce anesthesia, which was maintained for 4 hr by a second similar injection. Anesthetic controls were given equivalent amounts of pure olive oil over the 4-hr period. One hour after the conclusion of the anesthetic interval, rats in each treatment category were paralyzed by an ip injection of zoxazolamine (100 mg/kg).

[b] Values are recorded as mean time ± SE.

[c] Significantly longer than the phenobarbital–olive oil control level by the Student's t test ($p < 0.02$).

[d] *Not* significantly different from the saline–olive oil control level by the Student's t test.

ity, it seems reasonable to consider that this prolongation of paralysis time probably reflected diminished drug metabolizing capacity in the phenobarbital–halothane-treated animals. Davis *et al.* (19), however, were unable to demonstrate any significant effect of halothane upon the hepatic microsomal enzyme activities of phenobarbital-pretreated rats. The reasons for the difference between their results and those reported here are obscure, but might be related to dose or time factors. For example, the animals of Davis *et al.* (19) apparently were sacrificed at 24 hr after the administration of halothane, whereas our animals were tested soon after the conclusion of the anesthetic interval.

The present evidence of functional impairment of the liver was corroborated by the histologic study. Thus, at 24 hr after halothane anesthesia, the livers of *all* phenobarbital-pretreated rats displayed multiple, discrete foci of parenchymal necrosis (Fig. 1), and many also exhibited patchy subcapsular damage (Fig. 2). Both lesions were more prominent after 4 or 8 hr of anesthesia than after 2 hr. It should be emphasized, however, that, even after the longest anesthetic interval, the bulk of the hepatic parenchyma in these rats was morphologically intact. The histologic pattern was *not* that of massive hepatic necrosis; therefore, the relationship of this experimental injury to human posthalothane hepatitis is problematical. Notwithstanding, the experimental lesions described here are remarkably similar to those reported by Uzunalimoglu *et al.* (15) in *mild* cases of human posthalothane hepatitis.

In the present study, administration of halothane after saline pretreatment caused neither necrosis nor functional impairment of the liver at any of the anesthetic intervals tested. *Only* in the phenobarbital-pretreated rats did halothane cause liver damage. Phenobarbital is a well-known inducer of hepatic microsomal drug-metabolizing enzyme systems, and it is such enhancement that has been held accountable for the potentiation of carbon tetrachloride hepatotoxicity by phenobarbital pretreatment (28–30). Moreover, enhanced hepatic drug-metabolizing activity recently has been considered a vital factor in the development of massive hepatic necrosis in man after exposure to the halogenated anesthetic, fluroxene (36). Since halothane is metabolized to some extent in the liver (20–23) and since induction of hepatic enzymes increases this metabolism (24), one could speculate that enzyme induction was related to the liver damage observed in our phenobarbital–halothane-treated rats. We can, however, offer no direct proof of this, and it is clear that other factors, such as anoxia or disturbed hepatic blood flow (11), might have contributed to the observed liver injury.

Summary. Administration of halothane ip to rats pretreated with phenobarbital caused hepatic functional impairment in the immediate postanesthetic period. This impairment was manifested by a significant reduction of hepatic microsomal cytochrome P-450 levels and by a significant prolongation of zoxazolamine induced paralysis times. Both changes were noted in comparison with anesthetic control values. Similar phenobarbi-

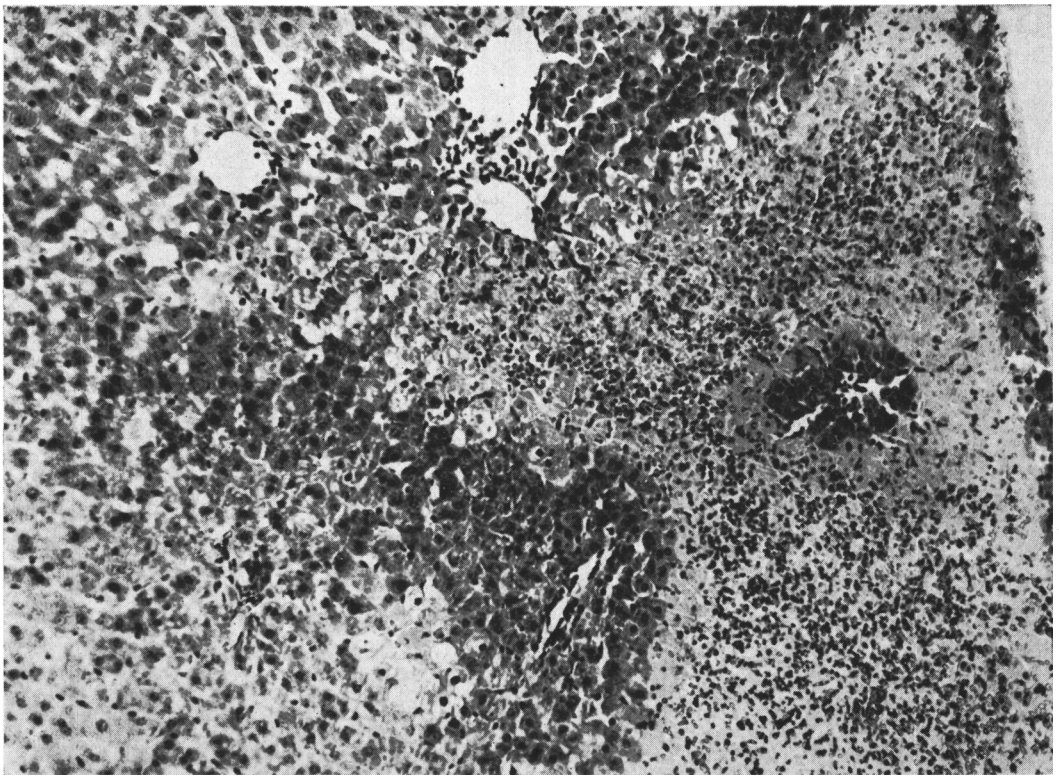

FIG. 2. Light micrograph illustrating patchy subcapsular necrosis of the liver. This rat was pretreated with phenobarbital for 3 successive days, anesthetized with halothane for 4 consecutive hr on Day 4, and killed on Day 5 to obtain the histologic sections. At the extreme upper right is the liver capsule, beneath which is a broad, irregular band of necrotic parenchyma that has been heavily infiltrated by inflammatory cells. A zone of condensed dark liver cells intervenes between the necrotic tissue on the right and the viable parenchyma on the left. ×150.

tal–halothane-treated animals were killed at 24 hr after anesthesia, and their livers were prepared for histologic evaluation. These livers invariably revealed multiple, discrete foci of parenchymal necrosis; many also displayed patchy subcapsular necrosis, as well. Equal amounts of halothane anesthesia produced neither functional impairment nor hepatic necrosis in rats pretreated with saline instead of phenobarbital.

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