Intraperitoneal Halothane Administration: Evidence of Hepatic and Muscle Injury[1] (35967)

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Serious hepatic injury has occurred in a very small proportion of patients anesthetized with halothane. The low incidence and clinical features of reported cases suggest that the injury reflects hypersensitivity to the agent rather than "true" hepatotoxicity (1, 2). This is also supported by the observation that administration of halothane by inhalation to experimental animals leads to little or no hepatic injury. Administration of halothane by another route (intragastric instillation) however, has been found to produce hepatic injury in experimental animals (3). Accordingly, in the effort to explore the potential hepatotoxicity of halothane further, the intraperitoneal route was selected for administration of this anesthetic agent.

Hepatic injury was monitored by assay of plasma levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), ornithine carbamoyl transferase (OCT), and sorbitol dehydrogenase (SDH). Plasma levels of creatine phosphokinase (CPK) also were measured, since recent studies have shown that halothane can induce muscle injury in genetically susceptible humans and pigs (4–7).

Materials and Methods. Female Sprague-Dawley rats, unfasted, weighing approximately 250 g, were studied. Halothane in the doses shown in Fig. 2 and 3 was administered by intraperitoneal (ip) injection. Blood was drawn in heparinized syringes from the abdominal aorta for assay of enzyme activity at 0.5, 1.5, 3, 6, 12, and 24 hr after the administration of halothane. Plasma was separated within 15 min and the assay was accomplished on the same day. Control animals received sodium pentobarbital (Nembutal, Abbott) or saline, ip, instead of halothane. In some experiments, the effect of diethylether, administered ip, was compared with that of halothane. The activity of the following enzymes was measured by the methods listed in the respective references: GOT, GPT (8), SDH (9), OCT (10), CPK (11), lactic dehydrogenase (LDH) (12), and LDH isoenzymes (13). Free hemoglobin of the plasma was measured by a standard cyanmethemoglobin assay (14). Analysis of variance and tests of significance were carried out according to Snedecor (15).

Results and Discussion. Administration of halothane in a dose of 3.5 mmoles/kg of body weight led to a sharp rise in the plasma levels of GOT, GPT, OCT, and to a slight, but significant, elevation in the level of SDH (Fig. 1 and 2). The values for GPT, OCT, and SDH were at or near their peak by 30 min and were back to normal by 12 hr (OCT, SDH) or 48 hr (GPT). Values for CPK and GOT reached a peak at 3 hr, returning to normal by 24 (CPK) or 48 hr (GOT). The relationship between the dose of halothane and degrees of hyperenzymemia is shown in Fig. 2. The degree of rise in levels of enzyme seemed proportional to the doses of halothane between 1.7 and 7.0 mmoles/kg of rat. At greater doses there was little or no increment. Evidence that the elevated enzyme levels reflect the agent administered rather than the route of administration or the effects of anesthesia is provided by comparing the effects of ether and of pentobarbital with those of halothane (Figs. 2 and 3). Intraperitoneal administration of pentobarbital in doses sufficient to induce anesthesia

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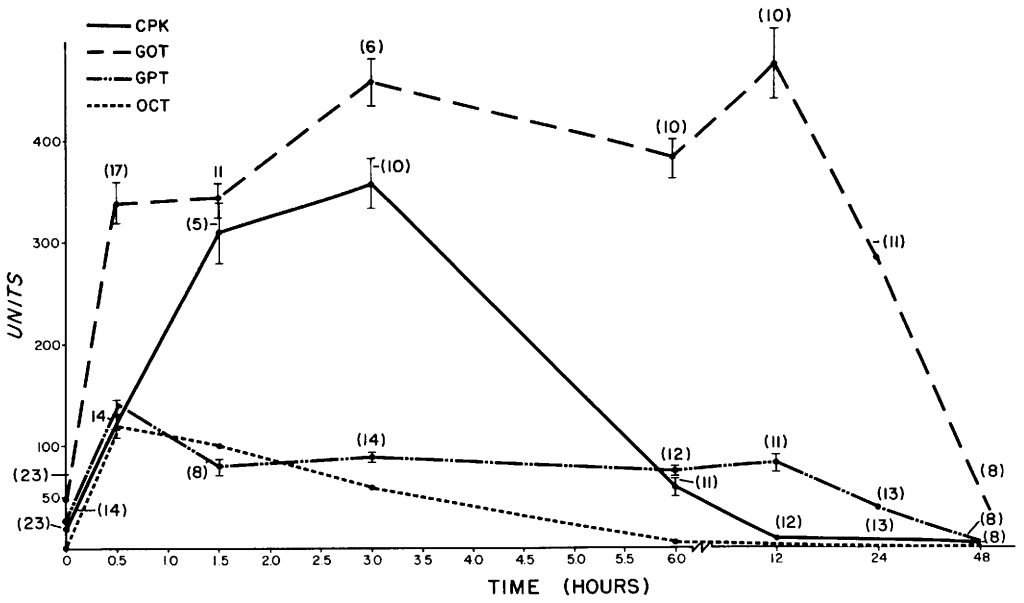

FIG. 1. Time course of plasma enzyme changes in rats after halothane administration: The dose was 3.5 mmoles/kg of body weight; (bars) standard errors of mean. Number of animals sacrificed to obtain each point is shown in parentheses. Values shown as "zero" time were taken before injection or at 24 or 48 hr after saline injection. Enzyme units are defined by the respective methods of assay.

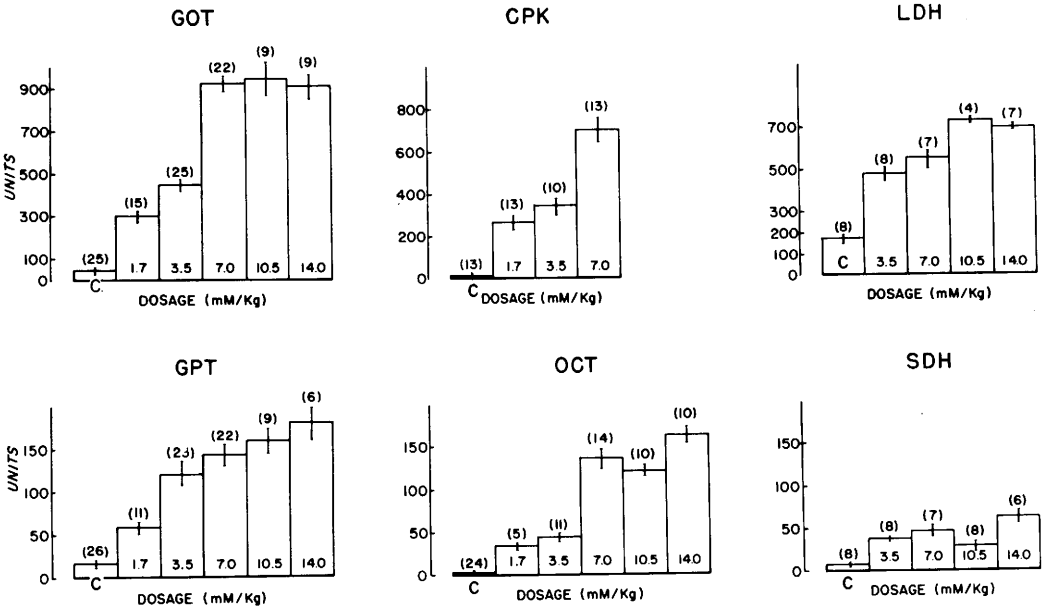

FIG. 2. Degree of hyperenzymemia after various doses of halothane (abscissa); (bars) standard errors of the mean. Controls (C) were treated with saline or sodium pentobarbital, or not treated at all. Assays were performed 3 hr after treatments. Number of animals used are shown in parentheses above each bar.

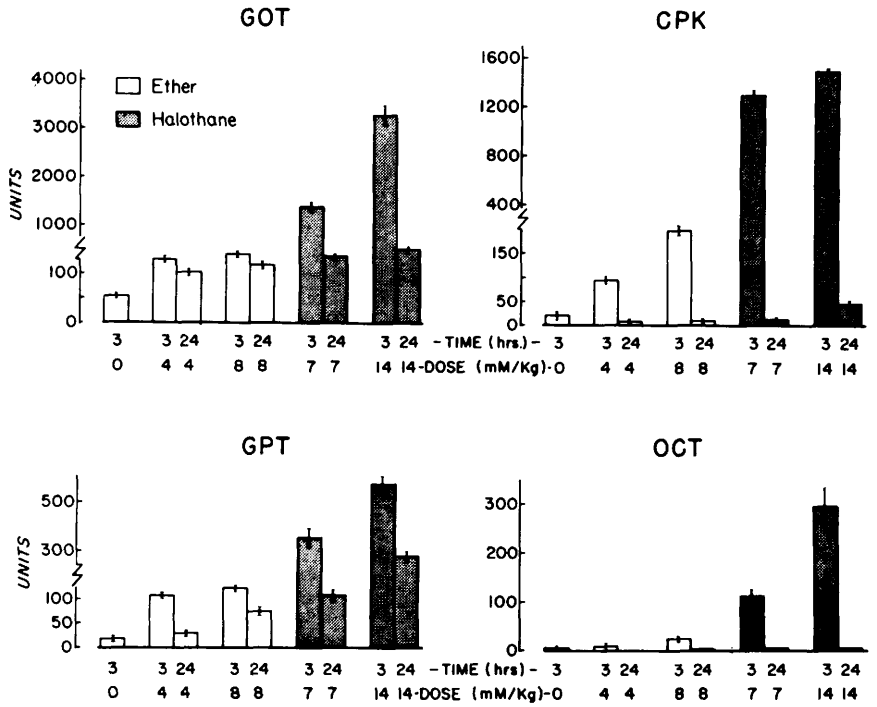

FIG. 3. Effects on plasma enzymes of halothane or diethyl ether administered to rats at times and in doses shown; (bars) standard errors of mean. Each treatment group consisted of 5 rats.

(0.25 mmoles/kg) led to values no different from those of saline-injected animals (Fig. 2) and untreated controls. Accordingly, the values for pentobarbital-treated, saline-injected and untreated controls were considered as a single group, labeled C in Fig. 2. The effects of administration of ether in doses of 4 and 8 mmoles/kg are shown in Fig. 3. Values for GOT, GPT, CPK, and OCT were significantly increased at 3 hr but to a significantly lesser degree than the levels produced by administration of halothane. By 24 hr, values for OCT and CPK were normal in ether-treated rats. GOT and GPT levels, at 24 hr, were still elevated but were significantly lower than in halothane-treated animals.

These data show that ip halothane leads to prompt enzymologic evidence of organ injury. High values in plasma of GOT and GPT may reflect hepatic injury, but the much greater elevation of GOT suggests that other tissues might have also contributed to the increased levels. The elevation of plasma CPK, and enzyme of which there is little or none in the liver, supports this view and suggests that skeletal muscle or myocardium might have been the source of some of the GOT and GPT. That liver damage was at least in part responsible for the elevated transaminase levels is suggested by the elevated levels of OCT and SDH.

A further effort to identify the possible organ source of the elevated enzyme levels was made by studying LDH isoenzymes (Fig. 4). Thirty minutes after administration of 3.5 mmoles/kg of halothane, the proportion of isoenzyme V was almost doubled with respect to saline-treated controls. These changes had reverted to almost normal at 3 hr after halothane treatment. Similar LDH isoenzyme patterns were apparent 3 hr after administration of 14 mmoles/kg of the anesthetic. These results are consistent with the other evidence of skeletal muscle and hepatic injury, and indicate that myocardial injury was not the source of the hyperenzymemia.

Slight evidence of hemolysis accompanied the hyperenzymemia and hemoglobinuria was occasionally observed at high doses of halothane. Free hemoglobin levels of plasma

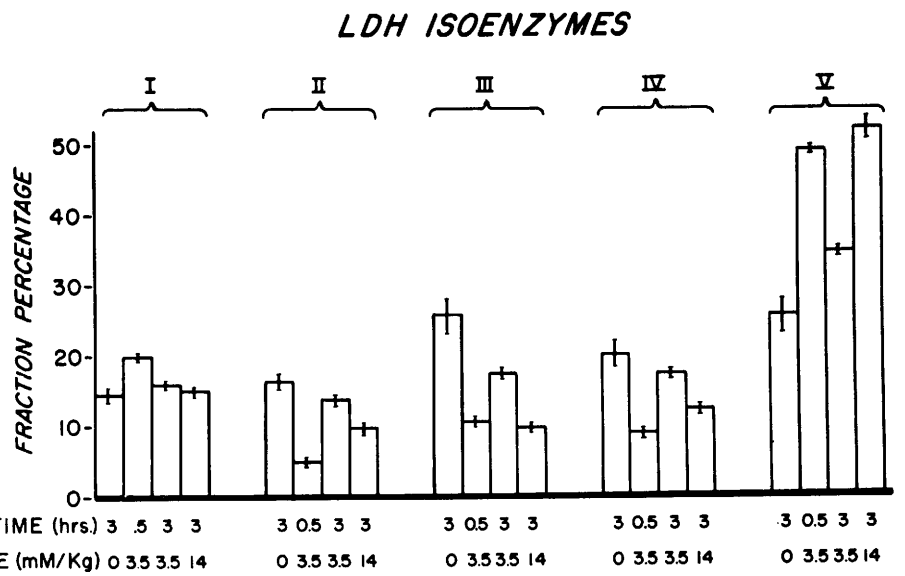

FIG. 4. Plasma LDH isoenzymes following treatment with halothane; six or more animals were used to obtain means; (bars) standard errors of mean.

rose to values as high as 700 mg/100 ml, representing a leakage of approximately 5% of the circulating hemoglobin. Only a small fraction of the elevated plasma levels of GOT, LDH, and CPK can be attributed to hemolysis. Furthermore, leakage from erythrocytes could not explain increased plasma levels of GPT, OCT, and SDH.

As shown in Table I, arterial blood gases (Po_2, Pco_2), arterial blood pH and bicarbonate content were measured[2] in control animals and in rats anesthetized with halothane (7.0 mmoles/kg) or pentobarbital (0.25 mmoles/kg), 30 to 45 min following ip injection of the test materials. The normal value for arterial oxygen tension obtained in the halothane-treated group is strong evidence against hypoxemia-induced organ injury in these animals.

These data demonstrate acute, transient injury to liver and muscle induced by intraperitoneal halothane administration. It is a dose-related hepatotoxic and myotoxic effect. The relevance of these observations to the likelihood of hepatic injury occurring after anesthetic administration of halothane by inhalation is open to question. Agents shown to be organotoxic by the ip route may not necessarily induce toxicity when administered by the respiratory route. The intrahepatic concentration of halothane achieved after ip injection is not known, but may be considerably higher than that attained by inhalational administration. Nevertheless, these observations demonstrate a dose-related hepatotoxicity that may be relevant to human hepatic injury even when it is apparently idiosyncratic. As has been suggested for other drugs (16), it is conceivable that halothane has a slight and transient effect on the liver which can be translated into frank hepatic necrosis when accompanied by hypersensitivity. The demonstration of muscle injury may be relevant to the recently described syndrome of "malignant hyperthermia (hyperpyrexia)" after anesthesia with halothane (4–7). Some of these studies have demonstrated elevated plasma CPK levels in susceptible patients as well as in a strain of susceptible pigs, in the absence of drug exposure. Plasma CPK elevations may be interpreted, in these instances, as a genetic marker of susceptibility to halothane-induced muscle injury. The observation of the present study, in another species (the rat) suggest that susceptibility

[2] The assays were carried out amperometrically in an automated pH blood gas analyzer No. 313, Instrumentation Labs., Lexington, MA.

TABLE I. Arterial Blood Gases, pH, Bicarbonate Content, and Plasma OCT in Rats Injected with Saline, Na–Pentobarbital or Halothane.

Treatment	*n*	P_{O_2}	P_{CO_2}	pH	HCO_3^-	OCT
Saline	5	75.6 ± 5	45.8 ± 2	7.388 ± 0.009	27 ± 0.8	0
Na–Pentobarbital (0.25 mmoles/kg)	5	65.8 ± 3	63.8 ± 5	7.250 ± 0.04	27 ± 0.6	0
Halothane (7 mmoles/kg)	4	81.7 ± 9	36.2 ± 2	7.340 ± 0.02	19 ± 1	160 ± 1

may prevail in the absence of a genetic marker (plasma CPK elevation) and may reflect a direct toxic effect of halothane.

Summary. Intraperitoneal administration of halothane, 1.7 to 14 mmoles/kg of body weight in rats, led to significant elevations in plasma levels of a number of enzymes (GOT, GPT, OCT, CPK, SDH, total LDH, and LDH isoenzyme V). This response was sharp and dose related. A similar but quantitatively lesser response was observed in rats treated intraperitoneally with ether. Normal plasma enzyme activity was found after injections of sodium pentobarbital or saline. These data suggest a direct, albeit transient, toxic effect of halothane on the liver and skeletal muscle of the rat.

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1. Little, D. M., *in* "Clinical Anaesthesia" (N. M. Greene, ed.), p. 95. Davis, Philadelphia (1968).

2. Klatskin, G., and Kimberg, D. V., N. Engl. J. Med. **280**, 515 (1969).

3. Jones, W. M., Margolis, G., and Stephen, C. R., Anesthesiology **19**, 719 (1958).

4. Kalow, W., Britt, B. A., Terreau, M. E., and Haist, C., Lancet **2**, 895 (1970).

5. Harrison, G. G., Saunders, L. J., Biebuyck, J. F., Hickman, R., Dent, D. M., Weaver, V., and Terreblanche, J., Brit. J. Anaesth. **41**, 844 (1969).

6. Woolf, N., Hall, L., Thorne, C., Down, M., and Walker, R., Brit. Med. J. **3**, 386 (1970).

7. Isaacs, H., and Barlow, M. B., Brit. Med. J. **1**, 275 (1970).

8. Zimmerman, H. J., and Henry, J. B., *in* "Clinical Diagnosis" (I. Davidsohn and J. B. Henry, eds.), 14th ed., p. 710. Saunders, Philadelphia (1969).

9. Gerlach, U., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 761. Academic Press, New York (1963).

10. Snodgrass, P. J., and Parry, D. J., J. Lab. Clin. Med. **73**, 940 (1969).

11. Sigma Tech. Bull. No. 661, Sigma Company, St. Louis, MO (1965).

12. Zimmerman, H. J., and Weinstein, H. J., J. Lab. Clin. Med. **48**, 607 (1956).

13. Gelman Instrument Co., Manual No. 70176-A, Ann Arbor, MI (1965).

14. Hycel Reagents, Houston, TX.

15. Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State Univ. Press, Ames (1956).

16. Zimmerman, H. J., Perspect. Biol. Med. **12**, 135 (1968).

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