Title: PENTANE EXPIRATION: A MEASURE OF HALOTHANE-INDUCED PEROXIDATION

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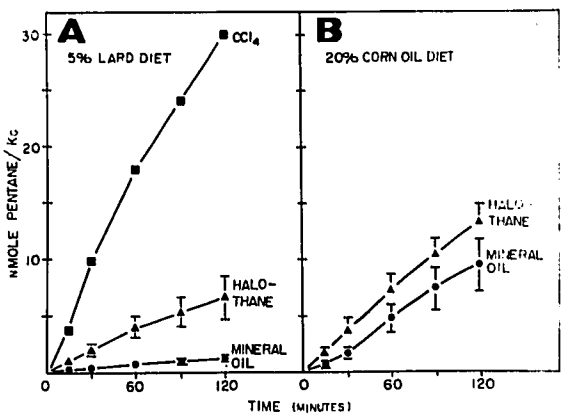
Introduction: Halothane hepatotoxicity in man is of infrequent and unpredictable occurrence. An understanding of the hepatotoxic mechanisms is paramount if procedures for predetermining susceptibility in patients are to be developed. Several mechanisms of toxicity have been proposed, one of which involves the peroxidative destruction of cellular membrane lipids. Halothane is metabolized by the hepatic microsomal mixed-function oxidase system to a free radical intermediate capable of initiating peroxidation. Recently, a non-invasive technique for the determination of lipid peroxidation in vivo was developed. This method measures volatile by-products of lipid peroxidation, pentane and ethane, in expired breath. An adaptation of this technique has been used to monitor lipid peroxidation in mice exposed to halothane.

Methods: Male CD1 mice were fed semi-purified diets containing the established minimum daily requirements of protein, vitamins, and minerals, and either 5% lard or 20% corn oil. Mice were injected i.p. with 6 mmole/kg of either halothane or CCl_4, or the mineral oil vehicle alone. They were subsequently placed in a 2.4 ℓ glass chamber through which purified air was passed. Samples of 5 ℓ air/breath were analyzed for pentane by gas liquid chromatography. Total liver microsomal heme content was determined 24 hrs after exposure from the characteristic difference spectra of cytochrome P_{450} and b_5.

Results: Mice treated with either halothane or CCl_4, a known hepatotoxin, significantly increased pentane expiration above that of control. For mice fed 5% lard diets, the cumulative pentane expiration was 1.00 ± 0.22, 6.64 ± 1.86, and 30.11 ± 10.91 nmole pentane/kg/120 min for mineral oil, halothane, and CCl_4, respectively (Fig. A). Pentane expired by mice fed a 20% corn oil diet was greater for both the halothane and mineral oil treatments, 13.30 ± 1.58 and 9.56 ± 2.28 nmole pentane/kg/120 min, respectively (Fig. B). The halothane dependent stimulation of pentane expiration, however, was less than that observed for mice fed the lard diet, 1.4- and 6.6-fold increases, respectively. Both cytochrome P_{450} and b_5 contents of liver microsomes decreased approximately 27% in the halothane-treated mice (Table). An equivalent dose of CCl_4 resulted in cytochrome P_{450} and b_5 contents of 0.227 ± 0.077 and 0.474 ± 0.195 nmole/mg protein, respectively. This represents a 64.9% decrease in cytochrome P_{450} but no significant change in b_5 content.



Effect of Halothane and CCl_4 on Pentane Expiration

EFFECT OF HALOTHANE ON LIVER MICROSOMAL HEMOPROTEINS

	MINERAL OIL	HALOTHANE	% DECREASE
5% LARD DIET			
CYT P_{450}	0.646 ± 0.154 (5)	0.454 ± 0.119** (5)	29.7
CYT b_5	0.380 ± 0.047 (5)	0.293 ± 0.089* (5)	22.8
20% CORN OIL DIET			
CYT P_{450}	0.963 ± 0.168 (3)	0.705 ± 0.165* (5)	26.9
CYT b_5	0.552 ± 0.036 (3)	0.395 ± 0.032** (5)	28.5

(N) = NUMBER OF ANIMALS
MEANS ± STANDARD DEVIATION; UNITS = NMOLE/MG PROTEIN
* $P < 0.05$; ** $P < 0.01$

Discussion: Halothane-dependent stimulation of lipid peroxidation in vivo has been demonstrated. Its magnitude is significantly less than that produced by the hepatotoxin, CCl_4. Pentane production was greater in corn oil-fed mice than in lard-fed, suggesting that pentane arose from peroxidation of ω-6 unsaturated fatty acids. Peroxidative damage to liver microsomal membranes is suggested by the decrease in cytochrome P_{450} and b_5 contents in response to halothane and CCl_4 treatments. The relationship between pentane evolution and hepatocyte necrosis is under current investigation in this laboratory.

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