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Massive Lethal Hepatic Necrosis in Rats Anesthetized with Fluroxene, after Microsomal Enzyme Induction

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Rats in which the level of cytochrome P450 is raised by phenobarbital pretreatment die of massive hepatic necrosis when exposed to fluroxene anesthesia. This observation has great relevance to man when consideration is given to the great number of commonly used therapeutic agents which induce increased levels of cytochrome P450. Fluroxene anesthesia should not be used for any patient taking such drugs. (Key words: Enzyme induction; Fluroxene; Liver, necrosis; Phenobarbital.)

UNTIL 1964, inhalational anesthetics in common clinical use, with the exception of trichloroethylene,[1] were considered biologically inert though pharmacologically active. In that year the publication by Van Dyke and associates[2] of their fundamental discovery that all inhalational anesthetics in common use were biotransformed, immediately allowed the possibility that mechanisms of anesthetic toxicity in the liver might have a biochemical basis not previously considered. Subsequent studies by the same workers[3-4] identified the endoplasmic reticulum of the liver cell as the site of, and the nonspecific hydroxylating enzyme cytochrome P450 as the agent for, these reactions.

A pilot study in the rat into the effects on liver function and histology of anesthetics administered in the presence of enhanced activity of this system[5] surprised us by revealing fulminant lethal hepatic necrosis in rats anesthetized with fluroxene, an anesthetic regarded until recently as innocuous to the liver.[6] We confirmed these findings in the following experiment.

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Method

Two groups of ten Wistar rats (female, 200–300 g) each were exposed to fluroxene anesthesia after the levels of cytochrome P450 in the livers had been increased by pretreatment with phenobarbital, ip.[7] The livers were subsequently examined histologically.

Group I rats were pretreated with phenobarbital, 50 mg/kg/day, ip for three days and thereafter (on day 4) anesthetized with fluroxene for one hour, this program being repeated three times. Survivors were sacrificed on the third day after the third anesthesia (fig. 1). Group II animals were pretreated with phenobarbital, 80 mg/kg/day, ip, for three days and thereafter (on day 4) anesthetized with fluroxene for three hours (fig. 2). Animals in both groups were starved for 24 hours before anesthesia (day 3), having been allowed food (Epol Balanced Rations) and water *ad lib.* at all other times.

With each of these primary experimental groups, three groups of control animals were associated (figs. 1 and 2). In the first control group (ten animals each) the two patterns of fluroxene anesthesia—1 hour × 3 or 3 hours × 1—with the 24-hour periods of preanesthetic starvation, were studied without phenobarbital pretreatment. In the second control group (ten animals each) the two schedules of phenobarbital administration (50 mg/kg/day and 80 mg/kg/day) were studied without exposure to fluroxene anesthesia. After sacrifice at the relevant time, the livers were examined histologically. A third group of animals (15 and 5) was used for estimation of the cytochrome P450 levels achieved by the two schedules of phenobarbital administration. In these animals cytochrome P450 content of liver homogenates was measured after the two schedules of phenobarbital ad-

GROUP 1

PROGRAM		NO. OF RATS	DAY: 1 – 3		4	---	15
ENZYME INDUCTION + FLUROXENE 1 HOUR		10	Ph B 50 mg/kg/day	STARVE	FLUROXENE 1 HOUR	X3	SACRIFICE
CONTROL	FLUROXENE 1 HOUR	10	–				
CONTROL	ENZYME INDUCTN.	10	Ph B 50 mg/kg/day		–		
CONTROL	CYT. P450 LEVEL	15	Ph B 50 mg/kg/day		sacrifice 5	sacrifice 5	sacrifice 5

FIG. 1. Group I program.

GROUP 2

PROGRAM		NO. OF RATS	DAY: 1 – 3		4	---	8
ENZYME INDUCTION + FLUROXENE 3 HOURS		10	Ph B 80 mg/kg/day	STARVE	FLUROXENE 3 HOURS		SACRIFICE
CONTROL	FLUROXENE 3 HOURS	10	–				
CONTROL	ENZYME INDUCTN.	10	Ph B 80 mg/kg/day		–		
CONTROL	CYT P450 LEVEL	5	Ph B 80 mg/kg/day		sacrifice		

FIG. 2. Group II program.

ministration by the method of Schoene *et al.*[8] as modified by Blekkenhorst.[9] For comparison, normal hepatic cytochrome P450 content was estimated in 11 untreated rats (not reflected in tables 1 and 2) on the same diet and after 24 hours of starvation.

The rats were anesthetized in groups of five in a dessicator 28 cm in diameter. The total volume of the dessicator was 27.3 liters, 4 liters of which comprised the volume below the grid, which was filled with standard anesthetic soda lime. Fluroxene, 3 per cent, in oxygen, was passed through the dessicator at a flow rate of 5l/min. This concentration of fluroxene was just sufficient to maintain a light plane of anesthesia, control animals regaining consciousness 2–3 minutes after discontinuance of the anesthesia.

TABLE 1. Liver Weights in Group II, Expressed as Percentage of Body Weight

	Control, No Treatment	Phenobarbital, 80 mg/Day × 3 → Fluroxene, 3 Hours	Fluroxene, 3 Hours	Phenobarbital, 80 mg/Day × 3
Number of rats	5	10	10	5
Liver weight MEAN SD	2.5 ±0.12	5.45 ±0.44	3.94 ±0.36	3.2 ±0.18

Results

All ten rats of Group II died during or immediately after their three-hour exposure to fluroxene, as did six of Group I, 12–24 hours after their first one-hour exposure. Their livers were enlarged (table 1) and showed massive central and midzonal necrosis (figs. 3, 4, and 5). Four Group I animals, though clinically sick after their first exposure to fluroxene, recovered and survived two further one-hour exposures.

Liver cytochrome P450 content was increased from control "no-treatment" levels by both schedules of phenobarbital pretreatment (table 2). The Group I schedule (50 mg/kg/day) increased cytochrome P450 levels by a factor (on average) of 1.5. The Group II schedule (80 mg/kg/day) increased cytochrome P450 content by a factor of 2.6 (difference statistically significant).

No rat in the control groups anesthetized with fluroxene without enzyme induction died, but the livers all showed demonstrable histologic changes (fig. 6), the most characteristic being feathery degeneration of the cytoplasm with widespread vacuolation.

The livers of rats pretreated with phenobarbital without exposure to fluroxene showed only the changes in liver weight (table 1) and histology described previously by Burger and Herdson.[10]

Discussion

This study shows that fluroxene anesthesia in the rat in the presence of increased levels of hepatic cytochrome P450 causes a rapidly lethal fulminant hepatic necrosis, and that fluroxene anesthesia alone causes histologically detectable changes in the liver. This is indeed a remarkable finding, considering that this drug was originally introduced into clinical practice as long ago as 1953,[11] that in subsequent clinical use in man it has accumulated an impressive record of clinical safety, and that, let alone there being any suggestion of hepatotoxicity, it has been recommended as the anesthetic of choice for hepatic[12] and renal[13] transplantation. Because of this one might seriously question the relevance to man of these findings in the rat, were it not for the recently published report[14] of the death from massive hepatic

TABLE 2. Cytochrome P450 after Phenobarbital Pretreatment

	Control, No Treatment	Group I			Phenobarbital, 80 mg/Day for 3 Days ×1
		Phenobarbital, 50 mg/Day for 3 Days ×1	Phenobarbital, 50 mg/Day for 3 Days ×2	Phenobarbital, 50 mg/Day for 3 Days ×3	
Number of rats	11	5	5	5	5
Cytochrome, P450, nM/g liver MEAN SD	44 ±7.9	70 ±35.0	62 ±5.9	70 ±19.4	116 ±28.7

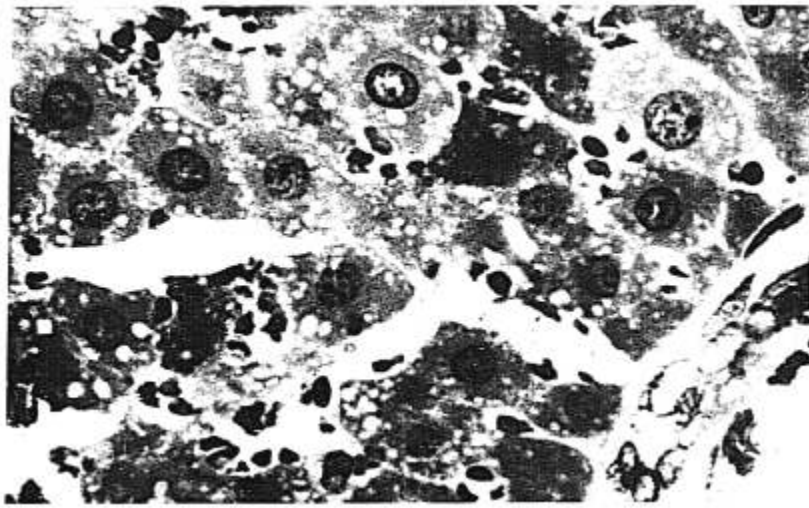

FIG. 3. Section of the liver of a Group II rat dying after 3 hours of exposure to fluroxene after enzyme induction, central vein at lower right. Note the cloudy swelling and acute early fatty infiltration. Two already-dead cells with darkly staining nuclei and dark cytoplasm are visible to right of center. Hematoxylin and eosin, ×400.

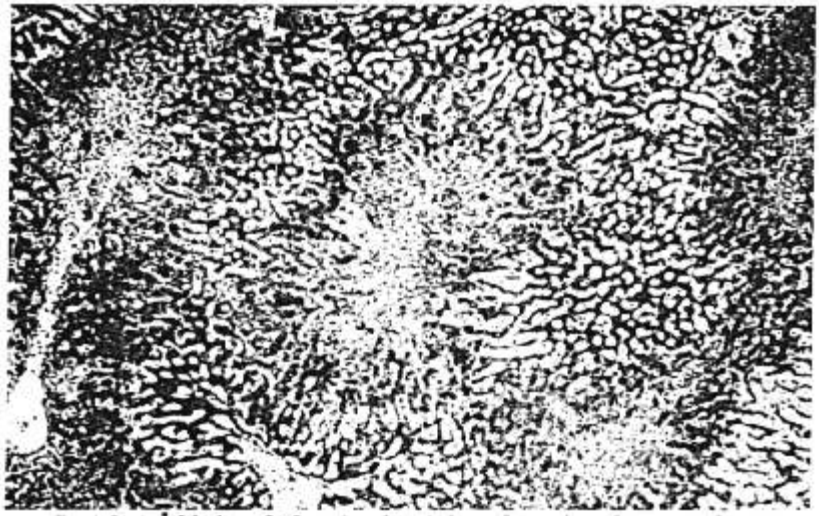

FIG. 4. Section of the liver of a Group I rat dying 24 hours after one hour of exposure to fluroxene after enzyme induction. Note the central and midzonal necrosis with dilated peripheral sinusoids. Hematoxylin and eosin, ×40.

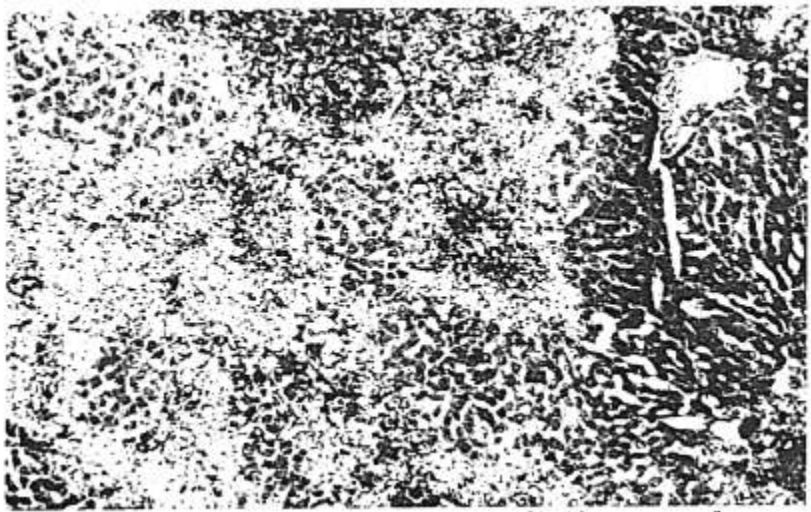

FIG. 5. Section of the liver of a Group I rat surviving a program of 3 × 1 hour exposures to fluroxene after enzyme induction. The rat was sacrificed on the third day after the last exposure. Note the complete central and midzonal necrosis with survival of peripheral zone cells. Hematoxylin and eosin, ×40.

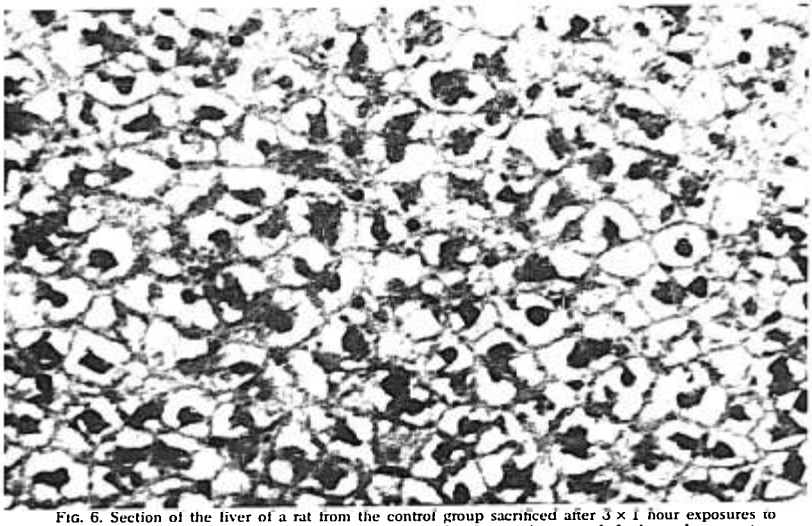

FIG. 6. Section of the liver of a rat from the control group sacrificed after 3 × 1 hour exposures to fluroxene without enzyme induction. Note the generalized vacuolation and feathery degeneration, giving a "chicken-wire" appearance. Hematoxylin and eosin, ×200.

TABLE 3. Some Drugs with Enzyme-inducing Properties

Hypnotics and sedatives	Barbiturates
Central nervous system stimulants	Amphetamines
Anticonvulsants	Meprobamate
	Chlordiazepoxide
Antipsychotics	Chlorpromazine
Hypoglycemic agents	Tolbutamide
Anti-inflammatory agents	Phenylbutazone
Antihistamines	Diphenylhydramine
Steroid hormones	

necrosis following fluroxene anesthesia for a gastrectomy of an epileptic patient treated with phenobarbital and diphenylhydantoin, drugs with potent enzyme-inducing properties.

The more fundamental question posed by our observations relates to the mechanism of hepatic damage and its relationship, if any, to that which follows other drugs such as carbon tetrachloride and chloroform, and to the whimsical hepatotoxicity of halothane.

Fluroxene is subject to biotransformation in the liver.[15-16] Evidence from our study that massive necrosis of the liver occurred only in animals in which fluroxene biotransformation would have been increased fits well with the observations of Cascorbi and Singh-Amaranath[17] that toxicity of fluroxene in mice was enhanced by enzyme induction and decreased by enzyme depression. All this evidence would seem to point to the probability that it is some reaction in or metabolite resulting from the biotransformation of fluroxene which is the toxic agent.

What this reaction or metabolite may be is a matter of conjecture. Though Blake and co-workers[18] have shown that trifluorethanol and trifluoroacetic acid, both products of fluroxene transformation, are toxic in dogs and mice, their aminals did not show any hepatic necrosis, although mild cloudy swelling and fatty accumulation are described as occurring in the dogs. To explain the gross hepatic necrosis we observed, we speculate that free radical formation[19] or epoxidation of the vinyl radical[20] of fluroxene as a step in its transformation to CO_2 may take place and result in lipid peroxidation of the cell organelle membranes, this leading ultimately to the massive cell destruction we saw.

Speculation aside, the clinical implication of our observations is clear. Fluroxene anesthesia should not be used for any patient who is on a regime of treatment with a drug that has enzyme-inducing properties[7] (table 3).

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References

1. Powell JF: Trichlorethylene absorption, elimination and metabolites. Br J Ind Med 2: 142–145, 1945
2. Van Dyke RA, Chenoweth MB, Van Poznak A: Metabolism of volatile anaesthetics. 1. Conversion *in vivo* of several anaesthetics to $C^{14}O_2$ and chloride. Biochem Pharmacol 13: 1239–1247, 1964
3. Van Dyke RA, Chenoweth MB: The metabolism of volatile anaesthetics. II. *In vitro* metabolism of methoxyflurane and halothane in rat liver slices and cell fractions. Biochem Pharmacol 14:603–609, 1965
4. Van Dyke RA: The metabolism of volatile anaesthetics. III. Induction of microsomal dechlorinating ether cleaving enzymes. J Pharmacol Exp Ther 154:364–369, 1966
5. Smith JS, Harrison GG: The effects of multiple anaesthetics on the livers of rats subjected to microsomal enzyme induction. S Afr Med J 47:797–799, 1973
6. Brechner VL, Watanabe RS, Dornette WHC: Values for SGOT following anaesthesia with Fluoromar. Anesth Analg (Cleve) 37:257–262, 1958
7. Conney AH: Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 19:317–355, 1967
8. Schoene B, Fleishmann RA, Remmer H, et al: Determination of drug metabolising enzymes in needle biopsies of human liver. Eur J Pharmacol 4:65–75, 1972
9. Blekkenhorst G: Personal communication, 1972
10. Burger PC, Herdson PB: Phenobarbital induced fine structural changes in rat liver. Am J Pathol 48:793–803, 1966
11. Kranz JC, Carr CJ, Lu G, et al: The anaesthetic action of trifluorethylvinyl ether. J Pharmacol Exp Ther 108:488–495, 1953
12. Aldrete JA: Anesthesia and intraoperative care, Experience in Hepatic Transplantation. Edited by TE Starzl, LW Putnam. Philadelphia, W. B. Saunders, 1969, p 96

13. Vetten KB: Anaesthesia for renal transplant. S Afr Med J 44:437–444, 1970
14. Reynolds ES, Brown BR, Vandam LD: Massive hepatic necrosis after fluroxene anaesthesia. N Engl J Med 286:530–531, 1972
15. Blake DA, Rozman RS, Cascorbi HF, et al: Biotransformation of fluroxene. Biochem Pharmacol 16:1237–1247, 1967
16. Blake DA, Cascorbi HF: A note on biotransformation of fluroxene. ANESTHESIOLOGY 32: 560, 1970
17. Cascorbi HF, Singh-Amaranath AV: Fluroxene toxicity in mice. ANESTHESIOLOGY 37:480–482, 1972
18. Blake DH, Cascorbi HF, Rozman RS: Animal toxicity of trifluorethanol and trifluoroacetic acid. Toxicol Appl Pharmacol 15:83–91, 1969
19. Slater TF: Necrogenic action of carbon tetrachloride in the rat. Nature 209:36–40, 1966
20. Udenfriend S: Arene oxide intermediates in enzymatic hydroxylation. Ann NY Acad Sci 179:295–301, 1971

Obstetrics

ARREST OF PREMATURE LABOR Adrenergic β-mimetic compounds are potent supressors of uterine contraction. However, these compounds also affect the cardiovascular system and may cause marked hypotension and tachycardia, thereby limiting their clinical usefulness. Ritodrine hydrochloride, a new β-mimetic compound, infused iv at rates of 100 to 300 μg/min, completely inhibited uterine contractility in five of six premature labors. Moderate increases in pulse pressure and heart rate occurred. Effective inhibition of the intensity of uterine contractions accompanied by minor cardiovascular changes would seem to make ritodrine valuable for the treatment of premature labor. (*Bieniarz, J., Motew, M., and Scommegna, A.: Uterine and Cardiovascular Effects of Ritodrine in Premature Labor. Obstet. Gynecol. 40: 65, 1972.*)

HYPOXIA AND FETAL HEART RATE Fetal bradycardia which commences during a uterine contraction and persists for 30 to 60 seconds *after* the contraction is over is called "late deceleration." This pattern of bradycardia during labor is usually associated with an infant severely asphyxiated and depressed at birth. An experimental model was developed in the subhuman primate to monitor directly the cardiovascular and acid–base state of the near-term fetus during labor. The relationship between late deceleration of the fetal heart rate, acid–base state, and level of oxygenation was studied in 30 experiments. Late deceleration was accompanied by a decrease in fetal oxygen levels. Fetuses well oxygenated during uterine contractions showed no change in heart rate or late deceleration. The latter was abolished or suppressed when fetal oxygenation was improved by administration of a high concentration of oxygen to the mother. (*James, L. S., and others: Mechanism of Late Deceleration of the Fetal Heart Rate. Am. J. Obstet. Gynecol. 113:578, 1972.*)