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# HEPATOTOXICITY OF INHALATION ANESTHETIC DRUGS

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CONTROVERSY concerning the effects of inhaled anesthetic agents on liver function has existed for many years (1, 2, 3). There are several reasons why differences of opinion have developed. First, it is realized that coexisting factors active in the organism may mislead one in the true interpretation of hepatotoxicity. Anoxia, the nutritional status of the subject, and alterations in hepatic blood flow are three examples of variables which may themselves contribute to liver damage (4, 5). Secondly, conclusions regarding toxicity to the liver which are based on the results of liver function tests are open to question because of the lack of specificity and accuracy of function reflected by such tests. Finally, employing conventional methods of administration, i.e., via the lungs, the concentration of drug exposed to the liver cells at any one time is difficult to determine and frequently is limited by the toxic effects of the drug on other vital organs of the body.

Some of these limitations became apparent while acute liver toxicity experiments relating to Fluothane were being carried out in dogs and monkeys (6). What was needed was a more direct approach to the problem, a method whereby a maximal concentration of drug could reach the portal circulation and hence the liver, and yet be compatible with survival of the animal. One of us (Margolis) suggested that the method of Morris and Thompson (7) for the hand feeding of mice might prove suitable. Eschenbrenner and Miller (8, 9) had already established that selective injury to the liver by such drugs as carbon tetrachloride and chloroform could be produced uniformly by this technique. This paper reports an adaptation of this method whereby the relative hepatotoxicity of several volatile inhalation anesthetics was

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determined. The drugs studied included chloroform, vinyl ether, ethyl ether, trichlorethylene and Fluothane. Owing to its known toxicity to the liver cell, chloroform served as a convenient point of reference to the other drugs.

## Method

Fifteen hundred white mice of a Swiss strain, sexes mixed, each approximately 20 Gm. in weight, were utilized. The animals were exposed in groups of ten to various concentrations of drugs. The anesthetics were administered orally according to the method of Morris and Thompson (7). An 18 gauge intravenous needle with a curved tip rendered blunt by an annealed platinum ball was passed into the esophagus. A tuberculin syringe graduated in 0.1 cc. increments was attached to the needle and contained the mixture to be instilled into the

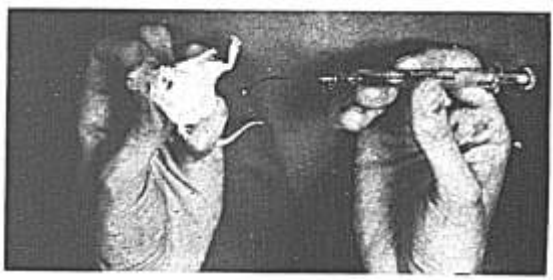

Fig. 1. Illustration of injection technique. Carefully measured amounts of drug were injected directly into the esophagus.

stomach (fig. 1). Each test drug was diluted to the concentration desired by mixing it on a volume/volume basis with olive oil. For example, if a 10 per cent solution was desired, one part of drug was mixed with 9 parts of olive oil. The quantity administered in each instance was 0.1 cc. Because of the volatility of some drugs, mixtures were made up immediately prior to use.

This method provided an easy means of introducing the anesthetic into the stomach and allowed primary absorption of the drug into the portal blood stream with concentration of its effect on the liver. A proper instillation of the drug produced onset of narcosis within five to ten minutes. An accidental pulmonary injection caused immediate onset of narcosis and quick death of the animal.

The mice were sacrificed 72 hours after exposure and the livers were formalin-fixed. Hematoxylin-eosin stains in paraffin embedded material were used for routine histologic study. Fat content was estimated from oil red stained frozen sections.

To determine whether lack of food intake after instillation of a

drug was a factor of significance in producing liver injury, two groups of 10 animals were tested. The amount of food intake (in the form of pellets) was measured in a control group which received olive oil only, and in one which received a concentration of chloroform known to produce hepatic necrosis. In the 72 hour period following instillation, the difference in food consumption between the two groups was insignificant.

In order to assess the possible role of extraneous factors such as climate, variation in batch lots and differences in feeding habits from month to month, a large-scale experiment was undertaken late in the investigation. In one day representative concentrations of drugs were administered to 25 groups of 10 animals each. The results of these experiments were compared with similar concentrations administered at other times to other batches of mice.

TABLE 1

SUMMARY OF THE RANGE OF CONCENTRATIONS OVER WHICH EACH DRUG WAS TESTED, AND THE NUMBER OF ANIMALS STUDIED WITH EACH AGENT

| Drug | Range of Concentrations | | Total Number of Animals |
|---|---|---|---|
| | Per Cent | Mg./Gm. | |
| Chloroform | 0.1–15 | 0.007–1.1 | 350 |
| Fluothane | 1–100 | 0.09 –9.3 | 350 |
| Trichlorethylene | 5–60 | 0.36 –4.3 | 200 |
| Vinyl ether | 10–50 | 0.34 –1.92 | 300 |
| Ethyl ether | 20–100 | 0.71 –3.6 | 300 |

## RESULTS

Utilizing this method of gastric administration it was possible to estimate for each drug tested the minimal narcotic dose ($MND_{50}$), the minimal lethal dose ($MLD_{50}$), the dosage which produced threshold hepatotoxic effects ($MTD_{50}$), and the degree of liver injury associated with progressively increasing concentrations of drug.

The range of concentrations tested for each drug is shown in table 1. The dosages are expressed as percentage of drug in the drug/olive oil mixture and in milligrams of drug per gram of mouse. The latter figure was calculated from the specific gravity of each drug in the following manner:

Example: S.G. of 1 cc. $CHCl_3$ (100 per cent) = 1.47 (1,470 mg.)
0.1 cc. $CHCl_3$ (100 per cent) = 147 mg.
0.01 cc. $CHCl_3$ (10 per cent) = 14.7 mg.
Average weight of mice = 20 Gm.
10 per cent $CHCl_3$ = 14.7/20 = 0.73 mg. per gram of mouse

In table 2 the drugs examined are listed in order of decreasing anesthetic potency. It was observed that chloroform and Fluothane

TABLE 2

SUMMARY OF DOSAGES REQUIRED FOR NARCOSIS, LETHAL EFFECT, AND MINIMAL HEPATOTOXIC EFFECT

| Drug | Minimal Narcotizing Dose (MND-50) | | Minimal Lethal Dose (MLD-50) | | Minimal Hepatotoxic Dose (MTD-50) | |
|---|---|---|---|---|---|---|
| | Per Cent | Mg./Gm. | Per Cent | Mg./Gm. | Per Cent | Mg./Gm. |
| Chloroform | 5 | (0.35) | 15 | (1.1) | 0.5 | (0.035) |
| Fluothane | 5 | (0.45) | 100 | (9.3) | 2 | (0.18) |
| Trichlorethylene | 10 | (0.72) | 40 | (2.92) | 10 | (0.72) |
| Vinyl ether | 20 | (0.68) | 40 | (1.36) | 20 | (0.68) |
| Ethyl ether | 30 | (1.07) | 80 | (2.84) | 80 | (2.84) |

produced narcosis at the lowest concentrations (column 1), while ethyl ether required the highest concentration. This sequence is in keeping with the known clinical potencies of the drugs. With optimum anesthetic concentrations the animals became unconscious and non-reactive to stimuli within five to ten minutes after administration of the drug. They remained in this state for approximately thirty minutes and then rather quickly recovered their normal activities and took an interest in food.

The minimal lethal concentrations of the several drugs are listed in table 2, column 2. In this study chloroform was the most lethal compound, while animals survived with exposure to 100 per cent Fluothane. Failure to survive was manifested at two intervals following instillation of the drug. Some animals died during the period of narcosis subsequent to drug administration. These deaths appeared to be related to respiratory failure. Most of the remaining mortalities occurred 24 to 48 hours after instillation of either vinyl ether or chloroform. Presumably the cause of death in these instances was related to severe liver injury.

TABLE 3

SUMMARY OF DEGREE OF LIVER INJURY ASSOCIATED WITH PROGRESSIVELY INCREASING CONCENTRATIONS OF DRUG

| Drug | Minimal Changes | | | | Moderate Changes | | Severe Changes | |
|---|---|---|---|---|---|---|---|---|
| | Midzonal Fatty Change | | Central Fatty Change | | | | | |
| | Per Cent | Mg./Gm. | Per Cent | Mg./Gm. | Per Cent | Mg./Gm. | Per Cent | Mg./Gm. |
| Chloroform | 0.5 | (0.035) | 1 | (0.07) | 2 | (0.14) | 5 | (0.35) |
| Fluothane | 2 | (0.18) | 10 | (0.9) | 40 | (3.6) | None observed | |
| Trichlor-ethylene | 10 | (0.72) | 12.5 | (0.91) | 20 | (1.46) | None observed | |
| Vinyl ether | None observed | | 20 | (0.68) | 25 | (0.85) | 35 | (1.19) |
| Ethyl ether | None observed | | 80 | (2.84) | None observed | | None observed | |

Dosages indicate lowest concentration at which these changes were observed.

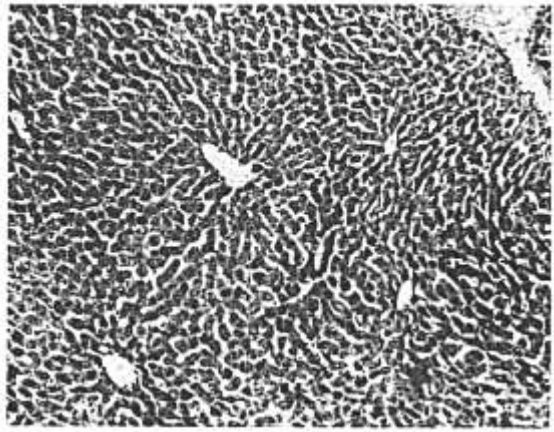

FIG. 2. Control liver section (olive oil), 72 hours after ingestion. Hematoxylin-eosin stain; × 141.

In table 3 are summarized the various dosage levels of drugs at which anatomic evidence of liver injury was observed. The results are graded on criteria which are based on the following histologic changes: (1) Minimal liver injury—mid-zonal fatty change, and mid-zonal plus

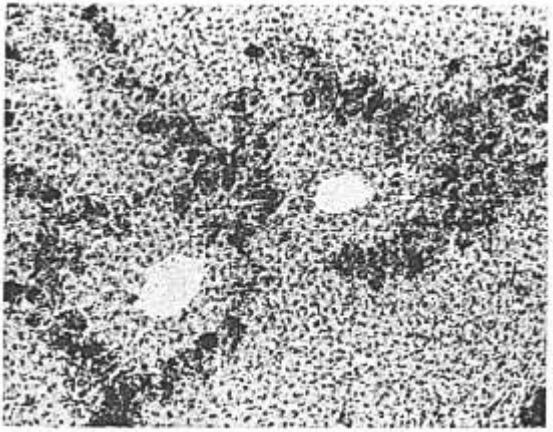

FIG. 3. Minimal fatty change in mid-zone of the liver lobule, 72 hours after ingestion of 0.5 per cent chloroform. Oil red stain; × 141.

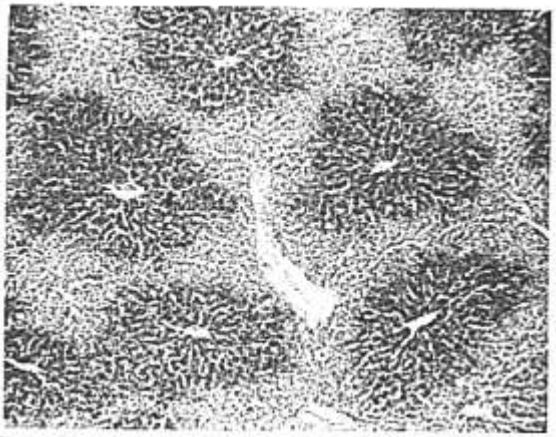

FIG. 4. Minimal fatty change in central zone of the liver lobule, 72 hours after ingestion of 10 per cent Fluothane. Oil red stain; × 81.5

centralobular fatty infiltration; (2) Moderate liver injury—massive fatty infiltration of total liver lobule, and (3) Severe liver injury—massive fatty infiltration with superimposed severe central necrosis of the lobule.

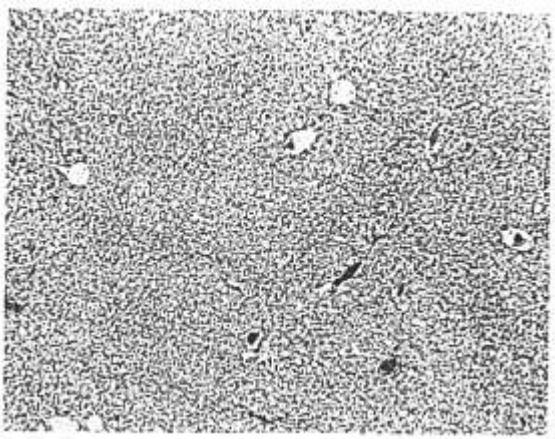

FIG. 5. Massive fatty infiltration of the liver lobule, 72 hours after ingestion of 100 per cent Fluothane. Hematoxylin-eosin stain; × 81.5.

The normal histologic appearance of the liver is shown in figure 2. This control animal received olive oil only. All drugs in the study produced at least minimal hepatotoxicity. Such changes are illustrated in figure 3, which shows mid-zonal fatty change with 0.5 per cent chloroform, and in figure 4, which shows central fatty change with 10 per cent Fluothane. While chloroform, Fluothane, trichlorethylene and vinyl ether all produced moderate liver cell injury, the latter three drugs required a considerably higher dosage than chloroform to do so (table 3 and figure 5). Only chloroform and vinyl ether produced severe liver injury in the form of frank necrosis (table 3 and figure 6).

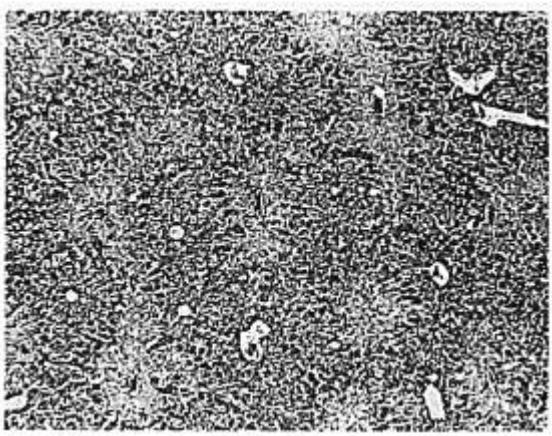

FIG. 6. Central necrosis of the liver lobule (pale areas), 72 hours after ingestion of 5 per cent chloroform. Hematoxylin-eosin stain; × 81.5.

In summary, the maximum liver injury which occurred with each drug, with survival of the animals, was as follows: *chloroform*—massive fatty change with centralobular necrosis, *vinyl ether*—massive fatty change with centralobular necrosis, *Fluothane*—massive fatty change, *trichlorethylene*—massive fatty change, and *ethyl ether*—minimal fatty change.

## Discussion

The experimental evaluation of systemic toxicity of drugs is a time-consuming and expensive procedure, requiring the use of large numbers of animals, and both acute and chronic toxicity studies. By concentrating on the acute hepatotoxic action of a group of anesthetic drugs, the present study has brought into focus a sensitive quantitative index which measures a single parameter of injury. The importance of the measured variable is indicated by the recognized hepatotoxic

effects of halogenated anesthetic drugs, and is emphasized by the severe degrees of liver injury encountered in this study. It is suggested that preliminary studies of this nature may be helpful in determining relative hepatotoxicity in the screening of new compounds.

The results obtained by the method of esophageal instillation reported herein serve as an index of the degree of hepatotoxicity to be expected in man. Although species differences may exist, it may be assumed that the mouse liver responds to drugs of this type in a manner similar to the liver of the human (10).

The rapid onset of narcosis, together with the progressive severity of lesions noted with increasing dosages, indicated that a large percentage of the drugs was absorbed from the gastrointestinal tract. This technique permitted the liver to be exposed to much higher concentrations of drugs, without death of the animal, than could be expected from administration by the respiratory route.

Attempts were made to rule out the possibility of extraneous factors influencing the degree of liver injury. It was shown that, during the 72 hour period following administration of a chloroform concentration which produced necrosis of the liver, the amount of food consumed by the inoculated group did not differ significantly from that eaten by a control group of mice. In the large-scale experiment performed on 250 mice in one day, the degree and extent of hepatic lesions noted with the various groups of animals were similar to those observed when the experiments were conducted on other occasions. However, it is possible that factors related to diet or nutrition may have contributed to the minimal hepatic lesions found.

Another factor to be considered in the etiology of the minimal liver changes is hypoxia. Oxygen was not administered to the animals during the period of narcosis following administration of the drugs. It is possible that drug-induced respiratory depression produced hypoxia. However, the rapid metabolism and recovery to normal feeding habits of the animals would minimize this possibility. The work of Richard with thiopental showed no differences in mice exposed to room air or extra oxygen, as far as hepatotoxicity was concerned (11).

An interesting observation pertained to the potent anesthetic properties of Fluothane, while at the same time administration of 100 per cent concentrations did not produce death of the animals (table 2). This particular finding was not in keeping with the trends seen with the other drugs.

According to the criteria employed, there were two types of minimal hepatotoxicity. The earliest injury seen with the halogenated drugs was a mid-zone fatty change in the liver lobule, which progressed to involve the central area with higher dosages. This mid-zone change has been described as the earliest and mildest manifestation of hepatic injury with chloroform in a recent detailed study by Myren (11). With the ethers this mid-zone change was absent and only a central pallor and vacuolation were seen.

In this study chloroform and vinyl ether were the most hepatotoxic drugs, both producing severe, necrotizing liver changes. Ethyl ether was relatively innocuous, a finding somewhat different to studies reported previously which relied on the Bromsulphalein excretion test (2). Perhaps the abnormalities of liver function reported clinically with ethyl ether are related more to its effects on metabolism in general than to a specific hepatotoxic action.

Fluothane, while capable of producing widespread fatty changes in the liver, failed to cause necrosis in the highest non-lethal dose employed, which was 100 per cent concentration.

## Summary

This study is presented as a laboratory screening method of evaluating relative hepatotoxicity in inhalation anesthetic drugs, using chloroform as a standard of reference.

Various concentrations of anesthetic drugs were administered to 1500 white mice by esophageal instillation. Histologic studies of the liver were made 72 hours after exposure to determine hepatotoxicity.

All drugs demonstrated at least minimal hepatotoxicity. Chloroform was the most toxic, ethyl ether the least toxic. Frank necrosis was observed with chloroform and vinyl ether only. Fluothane produced liver cell injury which was manifested as a fatty infiltration without necrosis.

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