THE EFFECT OF HALOTHANE ON THE LIVER OF DOGS EXPOSED TO MILD HYPOXIA¹

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HALOTHANE was reported by Raventós in his original report (1) to have no hepatotoxic effects in animals when used in concentrations up to 1.2 per cent in air or oxygen. The first clinical reports by Johnstone (2) made no mention of hepatotoxicity nor did those of Chang (3), Hudon (4), MacKay (5), Robson and Sheridan (6), and Abajian (7). A note of caution was sounded by Stephen *et al* (8). They reported that the chronic administration of halothane for a total of 24 hours spread over six days resulted in some central pallor in haematoxylin and eosin-stained sections of liver from dogs and monkeys. But they also stated that in humans halothane was less likely than other anaesthetic agents to produce liver dysfunction. Hall, Geisler, and Norris (9) reported the case of a patient exposed to a large amount of halothane with only slight congestion of liver and kidneys revealed at post-mortem examination. They felt that the drug had not contributed to the illness. Little and Barber (10) reported that halothane affected hepatic function less than cyclopropane or ether anaesthesia. Jones *et al.* (11) found halothane toxicity measured by intra-oesophageal instillation less than that of chloroform or divinyl ether, but greater than that of diethyl ether. Stephen *et al.* (12) conducted toxicity studies in dogs and reported a mild hepatotoxic action which again was much less than that of chloroform.

This study was undertaken to elucidate whether the combination of halothane with an inhaled gas mixture slightly deficient in oxygen might reveal any latent viscerotoxic properties of the agent, not apparent in the presence of adequate oxygen. Since mild degrees of hypoxia might occur during clinical anaesthesia for a variety of reasons, any significant effect of halothane under these conditions would markedly narrow its margin of safety.

METHOD

Four groups of mongrel dogs were studied. Each group consisted of six dogs weighing from 5 to 12.5 kg and groups were matched for weight. All dogs were premedicated with 15 mg of morphine and 0.6 mg. atropine intramuscularly at least 20 minutes prior to induction of anaesthesia. Induction was with 2.5 per cent sodium thiopental intravenously until the animals fell asleep, usually 25 to 30 mg./kg. This was supplemented as required with further thiopental while the dog was supine, the trachea intubated, a Silverman needle biopsy taken from the liver, and a femoral artery cutdown established. Mean arterial

¹Presented at the Western Division Meeting, Canadian Anaesthetists' Society at Saskatoon, March 19-21, 1959

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blood pressure was measured by means of a mercury manometer connected to the arterial cutdown. This system was flushed with heparinized saline from time to time.

Each dog was given 3 hours of inhalation anaesthesia. Those in the first group received halothane with 20 per cent oxygen and 80 per cent nitrous oxide. The second group received halothane in 100 per cent oxygen. The third group was given halothane in 15 per cent oxygen and 85 per cent nitrous oxide and the last group received 15 per cent oxygen and 85 per cent nitrous oxide alone. Nitrous oxide and oxygen were delivered from a McKesson anaesthesia machine to the endotracheal tube through a Fink non-rebreathing valve. Halothane was delivered from a Fluotec vaporizer with concentrations adjusted in such a way that the mean arterial blood pressure did not fall below 100 mm. Hg. This resulted in a mean concentration of halothane of 0.8 per cent. When respiration became depressed, it was controlled by means of a Stephenson ventilator. This was set to deliver 15 mm. Hg pressure at inhalation, no negative pressure being possible with a non-rebreathing valve.

At the conclusion of 3 hours of anaesthesia, a second needle biopsy was taken from the liver and the cutdown and endotracheal tube were removed. In most cases the dogs were struggling by this time and were taken to the recovery cages. They were fed *ad lib.* All animals in this series were alive and well next morning. At two and seven days postoperatively they were re-anaesthetized with intravenous thiopental and liver biopsies were taken.

The biopsy specimens were fixed in formaldehyde solution, embedded in paraffin and cut at 8μ . Sections from all blocks were stained with haematoxylin-eosin and with PAS (periodic acid-Schiff method); some sections were also stained with Heidenhain's iron-haematoxylin.

The preparations were examined microscopically without knowledge of the treatment in each case, but the pre-anaesthetic specimen was known as such and served as normal control. It should be kept in mind that, owing to the procedure of needle biopsy, the architectural pattern of the specimens becomes distorted.

From the aspect of the cell body in the haematoxylin-eosin preparation, in which most of the fat has been dissolved out by the embedding and straining procedures, the fat content was assessed and the sections graded in an arbitrary way (+, ++, +++, +++++). Microphotographs reproduced in Figure 1 illustrate two different aspects, one from animal C-1, the other from animal C-6. It appears quite reasonable to state that in the paler cells more material has either been dissolved out or has not accepted the aqueous staining solutions. This material could be either fat or glycogen, or both. We believe we are justified in assuming that the main substance responsible for the pale staining in the haematoxylin-eosin preparation is fat. This view is supported by an examination of alternate sections, that is sections from the same blocks, stained with PAS. If the paleness and vacuolization of liver cells in the haematoxylin-eosin preparation were caused by the loss of glycogen during fixation and staining, the paleness and vacuolization in the haematoxylin-eosin preparation would be paralleled by a decrease of staining intensity in the PAS preparation. This

FIGURE 1 Haematoxylin preparation of liver of animals No C-1 and C-6 (controls) Magnification $\times 106$ Compare size of liver cells in the two figures as well as the intensity of staining. It is assumed (see text) that the pale cells contain more fat than the darker staining cells.

was not the case and, therefore, it appeared safe to grade sections stained with haematoxylin-eosin for increase or depletion of fat content, and sections stained with PAS for glycogen content.

We are aware that this procedure is not the most accurate. The alternative was to fix half of each small specimen in formaldehyde solution and stain frozen sections with Sudan IV or Sudan Black, whereas the other half of the specimen would have to be fixed in absolute alcohol, embedded in paraffin and stained by Best's carmin method. The disadvantages of these procedures on the small tissue fragments, many of which had become brittle due to admixed blood from unavoidable haemorrhages outweigh, in our opinion, the advantages and the slight risk of misinterpreting small differences in fat and glycogen content.

RESULTS

Comparisons were made difficult by the fact that, as might be expected, there was a marked variation in the fat and glycogen content between the various control liver specimens (Figs 1, and 2). Hence, any changes must be interpreted as increases or decreases not from a common baseline but from the initial liver histology of any particular animal. Furthermore, the proximity or otherwise of the last feeding and a variable amount of ingested food before biopsy may show

up as mild changes in liver fat and glycogen. Hence, only marked differences between controls and later biopsies are significant.

The findings for fat and glycogen of the six animals in each of the four groups are graphically shown in Figure 2. The inconstant changes, usually within a narrow range, indicate that halothane has no more toxic effect on the dog liver

when administered in a hypoxic atmosphere than has the inhalation of the slightly hypoxic gas mixture alone. Indeed, if any trend is apparent at all, it is in the series of halothane in oxygen in which there was a consistent increase in the liver fat content. However, the average degree of this increase is still within the limits of normal variation and cannot be attributed therefore to the inhalation of halothane.

Except for one specimen, where there was what appeared to be a pathological vacuolization of liver cells, the body and nucleus of the parenchymal cells and of the sinusoidal cells showed no changes indicative of serious damage

SUMMARY AND CONCLUSIONS

Halothane has been shown to have no effect on the liver histology of dogs exposed to the drug for 3 hours while adequately ventilated. The agent has no adverse effect on liver fat, glycogen, and cellular structure even when it is administered in an atmosphere slightly deficient in oxygen.

ACKNOWLEDGMENTS

The authors are indebted to Dr. R. Altschul, Professor of Anatomy, who made the sections and interpreted them. Mr. K. Drummond, a medical student on an Abbott Research Grant, assisted with the investigations.

RÉSUMÉ

Nous avons étudié quatre séries de six chiens chacune, chiens à poids égal deux à deux. Chaque chien a été soumis à une anesthésie de trois heures. Un groupe a reçu de l'halothane avec 80 pour cent de protoxyde d'azote et 20 pour cent d'oxygène et un autre groupe a reçu de l'halothane avec oxygène seulement. Un troisième groupe a reçu de 85 pour cent de protoxyde d'azote et 15 pour cent d'oxygène sans halothane. Nous avons pris des biopsies hépatiques avant l'expérience et nous en avons pris d'autres immédiatement après, 24 heures plus tard et sept jours plus tard. Sur les coupes, on a étudié les changements hépatiques en graisse et en glycogène. Nous n'avons pas trouvé de changements importants et, en conséquence, nous pouvons conclure que, dans les conditions expérimentales données, l'halothane ne produit pas de dommages sérieux sur l'histologie du foie.

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