EFFECTS OF HALOTHANE AND CHLOROFORM ON THE LIVER
IN PROTEIN-DEFICIENT MICE

BY

DANIEL WINGARD,* HAMILTON S. DAVIS AND DONALD LEONARD

From the Departments of Anesthesiology and Pathology, Western Reserve University
School of Medicine, Cleveland, Ohio, U.S.A.

SUMMARY

A single 45-minute exposure to halothane did not cause demonstrable pathological liver
changes when administered to mice in an atmosphere of air or oxygen regardless of
whether the diet was normal or low in protein. Similarly, increased inhaled oxygen
tensions per se had no influence on liver changes. The well-known hepatotoxicity of
chloroform was demonstrated in groups fed both regular and low protein diets. The
animals tolerated the single chloroform anaesthesia less well than those receiving halo-
thane with significant liver damage in both groups.

Halothane† has been shown repeatedly in man
and in animals to have a benign effect upon the
liver (Brindle, Gilbert and Millar, 1957; Burns
et al., 1957; Carson et al., 1959; Gibson, 1959;
Green et al., 1959; Hansen, Davis and Hardy,
1959; Johnstone, 1956; Krantz et al., 1958; Little,
Barbour and Given, 1958; Morris, 1960; Quijani,
1958; Raventós, 1956; Virtue et al., 1958; Visser
and Tarrow, 1959). Although Jones, Margolis and
Stephen (1958) have produced massive fatty in-
filtration in mice following single intragastric
administration and Stephen and his associates
(1958) found centralobular fatty alteration
following repeated inhalation in dogs, these
changes were mild when compared with divinyl
ether and chloroform.

However, the majority of data has been obtained
from individuals receiving adequate diets and
sufficient oxygen. Oxygen deprivation and malnu-
trition singly and combined are known to enhance
the hepatotoxicity of many drugs including chloro-
form (Drill, 1952; Goldschmidt, Ravdin and
Lucke, 1937; Goldschmidt, Vars and Ravdin,
1939; Miller and Whipple, 1940. The possibility
remains that halothane, a halogenated hydrocarbon
like chloroform, might cause liver damage in the
presence of lowered oxygen tension and malnutri-
tion. Haley and Wyant (1959) found no histo-
logical changes in the liver of dogs when halothane
was administered in the presence of mild hypoxia
(oxygen 15 per cent and nitrous oxide 85 per cent
inhaled mixture).

Data available on the effects of halothane
administered in the presence of nutritional defi-
ciency are meagre. Virtue and associates (1958)
have shown that fasting dogs for several days
before anaesthesia does not adversely affect brom-
sulphathalein excretion following a 4-hour expo-
sure to halothane under mildly hypoxic conditions.
Previous studies in our laboratory using a protein-
poor diet showed an increased incidence of hepatic
cellular changes in mice after 4 weeks of this diet
alone and a further increase in incidence of such
changes following the addition of 1 per cent halo-
thane in oxygen (Davis, Leonard and Quitmeyer,
1961). On the other hand, humans with severe
burns, and presumably associated malnutrition,
have received halothane repeatedly without liver
damage (Hansen, Davis and Hardy, 1959; Quijani,
1958; Visser and Tarrow, 1959).

This study was devised to compare the effects
of halothane and chloroform upon the livers of
a group of mice receiving a normal diet and a
group receiving a low protein diet.
METHOD

Eleven hundred and one white male Swiss mice weighing 20 to 27 grams were boarded in individual cages. Although purchased from the same source* only enough animals were purchased at one time to complete studies on one group. Separate controls were thus necessary for each of the four groups in the study designated A, B, C and D. All were fed a regular diet† in pellet form and allowed to acclimatize for at least one week before tests were begun. Batches of 20-25 mice were anaesthetized at a time.

The chosen anaesthetic agents were administered for 45 minutes in a 100-litre Zauder-Orkin chamber (Zauder and Orkin, 1959). Halothane 0.86 per cent and 1 per cent and chloroform 1 per cent and 1.3 per cent were used. The amount of liquid agent necessary to give the desired vapour concentration was determined by standard calculations using the known physical properties of both agents.‡

Group A (380 mice) was fed a regular diet. Eighty animals were exposed to pure oxygen only for 45 minutes. Ninety-eight animals received halothane 1 per cent in oxygen; ninety-two received halothane 1 per cent in air. One hundred and ten control animals received no anaesthesia.

Group B (239 mice) was fed a regular diet. One hundred animals were anaesthetized with halothane 0.86 per cent in oxygen. Eighty animals were anaesthetized with chloroform 1.3 per cent in oxygen. Raventós (1956) reported these to be the equivalent AD/50 doses of the two drugs. Fifty-nine animals receiving no anaesthesia served as controls.

Group C (248 mice) was fed a regular diet. Ninety-nine mice were anaesthetized with halothane 1 per cent in oxygen and ninety-eight were anaesthetized with chloroform 1 per cent in oxygen. Fifty-one animals served as controls.

Group D (234 mice). Thirty-four control animals, fed a regular diet throughout the test period, received no anaesthesia. The remaining animals were fed a low protein diet containing 8 per cent casein for four weeks.* Sixty-seven of these animals received the diet alone, sixty-eight were in addition anaesthetized with halothane 1 per cent in oxygen and sixty-five received chloroform 1 per cent in oxygen.

Each group of test mice was divided into four approximately equal batches. The first batch was sacrificed a few minutes after anaesthesia by decapitation. Livers were excised immediately and fixed in formalin. Haematoxylin-eosin stained tissue sections were prepared.

The remaining three batches of animals were sacrificed similarly on the 2nd, 5th and 10th days after anaesthesia, the liver excised and fixed, and H-E slides prepared in a like manner.

Control animals were sacrificed at appropriate intervals during the tests and their livers studied by the same techniques.

The sections were examined as unknowns by the same pathologist (D.D.L.) in all instances. Slides were prepared by the same technician throughout and were identified only by numbers until completion of the study at which time the numbers were decoded.

RESULTS

Grossly, most livers appeared normal. Histological changes found included degeneration and necrosis.

Normal mouse livers (fig. 1) were characterized by even staining and normal appearing nuclei, portal areas and central veins.

Degenerative changes (fig. 2) were characterized by areas of pallor which were predominantly centralobular, by intracytoplasmic coagulation and by vacuolization. The nuclei were normal. Specific fatty changes were rarely found.

Necrosis (fig. 3) was characterized by loss of cellular outline and detail with abnormal or absent nuclei.

* Obtained from Rockland Farms, New City, Rockland County, New York.
† Rockland Mouse Diet (A. E. Staley Mfg. Co., Decatur, Ill.):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>24 per cent min.</td>
</tr>
<tr>
<td>Crude fat</td>
<td>4 per cent min.</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6 per cent min.</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>50 per cent min.</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>2,500 USP units/lb.</td>
</tr>
</tbody>
</table>

‡ 22,400 ml (STP) × specific gravity = ml vapour/ml liquid

gram molecular weight

* 8 per cent low protein diet (Nutritional Biochemicals Corp., Cleveland 28, Ohio):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein†</td>
<td>8 per cent</td>
</tr>
<tr>
<td>Starch</td>
<td>78 per cent</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>10 per cent</td>
</tr>
<tr>
<td>Salt mix USP XIV</td>
<td>4 per cent</td>
</tr>
</tbody>
</table>

† See Bodansky (1938) for amino acid composition.
EFFECTS OF HALOTHANE AND CHLOROFORM ON THE LIVER

FIG. 1
Normal mouse liver characterized by uniform staining of cytoplasm with normal appearing nuclei. The arrow points to a portal triad containing a vein, artery and bile duct. The triangle points to a central vein. (Magnification ×90, H & E stain.)

FIG. 2
Liver degeneration after chloroform 1% per cent and low protein diet. This slide shows two portal triads (one indicated by arrow) surrounded by normal appearing hepatic cells. Three central veins (one indicated by triangle) are present with surrounding areas of degeneration characterized by pallor, intracytoplasmic coagulation and vacuolization. Most of the nuclei are normal. (H & E stain, ×90 magnification.)

FIG. 3
Liver necrosis after chloroform 1% per cent and low protein diet. There are three portal triads (one indicated by an arrow) surrounded by normal appearing liver cells. There are five central veins (one indicated by triangle) surrounded by areas of necrosis characterized by pallor, loss of cellular outline and detail, with abnormal or absent nuclei. (H & E stain, ×90 magnification.)
**Table I**  
Effects on the liver of oxygen, halothane 1% in air and halothane 1% in oxygen in mice receiving a regular diet.*

<table>
<thead>
<tr>
<th>Day sacrificed post-anaesthesia</th>
<th>Controls: regular diet alone</th>
<th>100% oxygen for 45 minutes</th>
<th>Halothane 1% in air for 45 minutes</th>
<th>Halothane 1% in oxygen for 45 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. animals</td>
<td>Liver changes</td>
<td>Total</td>
<td>No. animals</td>
</tr>
<tr>
<td>0</td>
<td>35</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>110</td>
<td>7</td>
<td>3</td>
<td>10/110</td>
</tr>
</tbody>
</table>

*Rockland mouse diet (protein 24%)

**Table IV**  
Effects on the liver of halothane 1% and chloroform 1% in oxygen in mice receiving a low protein diet.*

<table>
<thead>
<tr>
<th>Day sacrificed post-anaesthesia</th>
<th>Controls: 8% protein diet for 4 weeks</th>
<th>8% protein diet for 4 weeks plus 45 min. of halothane 1% in oxygen</th>
<th>8% protein diet for 4 weeks plus 45 minutes of chloroform 1% in oxygen</th>
<th>Controls: regular diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. animals</td>
<td>Liver changes</td>
<td>Total</td>
<td>No. animals</td>
</tr>
<tr>
<td>0</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Totals</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>68</td>
</tr>
</tbody>
</table>

* Low 8% protein diet (casein 8%)
† These animals either died or were sacrificed at 36 hours (see text)
‡ Highly significant difference (P <0.001)
Group A (table I).
In no instance did halothane have any apparent effects upon the livers of mice fed a normal diet. The fact that there was a demonstrable, though small, incidence of degenerative changes and necrosis in these control groups emphasizes the absolute need for adequate controls in studies such as this. The data indicate that there were no differences related to anaesthesia between the test groups or between control and test groups when halothane 1 per cent was administered in air or oxygen. Further, there was no difference in the amount of liver damage found in the animals receiving pure oxygen only for 45 minutes.

**TABLE II**
Effects of halothane 0.86% in oxygen and chloroform 1.3% in oxygen on the livers of mice receiving a regular diet.*

<table>
<thead>
<tr>
<th>Day sacrificed post-anaesthesia</th>
<th>Controls: diet alone</th>
<th>Halothane 0.86% in oxygen for 45 minutes</th>
<th>Chloroform 1.3% in oxygen for 45 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. animals</td>
<td>Liver changes</td>
<td>Total</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>1 2 3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>1 0 1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>1 0 1</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>59</td>
<td>1 2 3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Regular Rockland diet (protein 24%)

**TABLE III**
Effects of halothane 1% and chloroform 1% in oxygen on the liver of mice receiving a regular diet.†

<table>
<thead>
<tr>
<th>Day sacrificed post-anaesthesia</th>
<th>Controls: diet alone</th>
<th>Halothane 1% in oxygen for 45 minutes</th>
<th>Chloroform 1% in oxygen for 45 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. animals</td>
<td>Liver changes</td>
<td>Total</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>2 1 3</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>2 0 2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1 0 1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4*</td>
<td>2 1 3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>2 1 3</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>51</td>
<td>2 1 3</td>
<td>3</td>
</tr>
</tbody>
</table>

* Animals found dead.
† Rockland mouse diet (protein 24%)
‡ \( \chi^2 = 4.36, P < 0.05 \)
Group B (table II).

Of the eighty animals originally used in the chloroform group, fourteen died without benefit of autopsy and are not included in the statistical analysis. Eight of the sixty-six mice autopsied showed degenerative changes in their livers but no necrosis. Using chi-square analysis, there was no significant difference from the controls ($P<0.20$). Three of the hundred mice receiving halothane had significant liver disease but this was not statistically different from the controls ($P=0.50$).

Group C (table III).

Halothane 1 per cent and chloroform 1 per cent was used in this group. Liver studies were obtained in all animals including those dying during the test period. Two animals in the control group died on the second and fourth days respectively but showed no evidence of liver damage. Two animals also died in the halothane group, again without evidence of liver damage.

Those in the group receiving chloroform 1 per cent did not do as well during the postanaesthetic period. Four animals dying during anaesthesia did not show liver changes. Of the eleven dying later, five showed significant liver damage; these five died between 24 and 72 hours after anaesthesia. The cause of death in the remaining six animals was not apparent.

There was a higher incidence of liver changes in both test groups than in the control group. Using chi-square analysis, the difference from controls was not significant in the halothane group ($P=0.30$); in the chloroform group the incidence of liver damage was significant ($P<0.05$).

Group D (table IV).

Thirty-four control animals fed a regular diet showed no liver damage. Further, four weeks on the low protein diet alone did not produce changes.

In the sixty-eight mice fed a protein-deficient diet, there was no liver damage with halothane 1 per cent in oxygen. By contrast, there was liver damage found in mice fed a low protein diet following chloroform 1 per cent in oxygen. There were no obvious lesions in the livers of animals killed immediately after anaesthesia. The remaining forty-seven animals in this batch did poorly. They never fully recovered from the anaesthetic, followed a progressively downhill course and became moribund, with many dying before 36 hours elapsed. These dead animals were autopsied within 2 hours of death. Because of the possibility of being unable to autopsy the remaining obviously moribund animals within a reasonable time postmortem, the entire batch was sacrificed at 36 hours. In this group, one showed fatty degeneration, twenty-nine showed the severe cytoplasmic changes described previously and three showed necrosis. The presence of hepatic changes in these thirty-three animals represented a difference from the controls which was statistically highly significant ($P<0.001$).

DISCUSSION

Previous experimental evidence indicates that halothane is not toxic to the liver of animals and men. The results of this study support the idea. On the other hand, chloroform has been shown repeatedly to have hepatotoxic effects. In this study the mice responded poorly to chloroform, developing liver damage in both normal and low protein diet groups.

The concentrations of halothane (0.86 per cent) and chloroform (1.3 per cent) used in Group B were chosen from the data of Raventós (1956). The activity of the animals during administration of the anaesthetic agents was similar, although those receiving chloroform appeared to be slightly more deeply anaesthetized. Because of the poor performance of the mice anaesthetized with 1.3 per cent chloroform, subsequent animals were anaesthetized with 1 per cent chloroform and 1 per cent halothane in order to favor chloroform. The mice appeared to be at the same level of anaesthesia when given these concentrations for 45 minutes. Mørch and Jobgen (1959) found that halothane and chloroform are equipotent when given to mice.

Animals were anaesthetized for 45 minutes for several reasons. The time interval was the same as that used by Gibson (1959) and, therefore, served as a comparison with his work. Also, this was the first in a series of studies of the effect of anaesthetics upon mouse livers. It was devised to determine the effects of halothane and chloroform upon the mouse liver after a moderate exposure.
The batches were limited to a maximum of twenty-five mice to prevent crowding and to minimize reduction of anaesthetic concentration within the chamber through uptake by the animals. It was recognized from preliminary work that mice frequently show the pathological liver changes of focal necrosis and periporal degeneration, even in apparently healthy mice. Therefore, it became evident that each group of mice must have its own control animals. For this reason, there was no overlapping of animals between experimental groups. All animals of each group were the same age and were obtained at the same time. It became apparent that the animals in Group D must have been healthier to start with than those in Groups A, B, and C.

No attempt was made to weigh individual animals as a means of assessing the state of nutrition. Animals on the low protein diet (8 per cent casein) were definitely more irritable than those on the regular diet and their fur lacked lustre. Osborne and Mendel (1960) showed that a diet with 9 per cent casein results in very poor growth when fed to rats and requires the addition of the amino acid, cystine, to support good growth. The low protein diet used during this experiment was similar to diets VII and VIII used by Goldschmidt, Vars and Ravidin (1939) in their classical studies on the influence of diet upon chloroform hepatotoxicity in rats. We were attempting to produce minimal effects with the deficient diet for it was recognized that severe changes caused by diet alone might well obscure changes due to subsequent anaesthetic effects. The results obtained with Group D animals receiving chloroform after a four-week low protein diet supports the premise that this diet per se did indeed have a deleterious effect upon the liver. On the other hand, the absence of fatty infiltration and fatty degeneration in these animals suggests that the liver damage from the diet was not severe.

The first portion of this study was aimed at testing the alleged protective action on the liver of an increased oxygen tension (Goldschmidt, Ravidin and Lucke, 1937). In preliminary studies in our laboratory (Davis, Leonard and Quitmeyer, 1961), not only was such a protective action absent but a surprising incidence of liver changes was found in animals 5 to 10 days after a single administration of halothane 1 per cent in oxygen but not in air. Also Haley and Wyant (1959) had found a consistent increase in the fat content in dog livers after the administration of halothane in oxygen whereas those receiving an hypoxic atmosphere did not show this change. The present study differed from our previous work in its failure to show pathological liver changes following exposure to oxygen alone. Nor did the addition of halothane to the oxygen in these experiments produce such changes even in the presence of low protein diet. Because of the demonstrated poor reaction of the mice on a regular diet to chloroform 1.3 per cent, as compared with those given the equivalent concentration of halothane 0.86 per cent, the bias was deliberately shifted in favour of chloroform for subsequent studies. Accordingly, the halothane concentration was increased to 1 per cent and the chloroform decreased to 1 per cent. Even then, halothane had no apparent deleterious effect upon the mice.

However, chloroform did have pronounced effects upon the mouse liver. There was a significantly higher incidence of damage in mice fed a regular diet especially in the 2- to 5-day post-anesthesia periods. Although the liver damage in Group B due to chloroform 1.3 per cent was not statistically significant, there was a suggestion that the chloroform was more toxic than the halothane. Also, it is quite likely that many of the livers in the fourteen animals in this group dying without benefit of autopsy would have demonstrated toxicity due to chloroform. Further, the animals fed a low protein diet did so poorly that all had either expired or were moribund by 36 hours after anaesthesia and had to be sacrificed prematurely. The incidence of marked liver damage in the latter group was highly significant when compared with controls.

REFERENCES


**BRITISH JOURNAL OF ANAESTHESIA**

**EFFETS DE L’HALOTHANE ET DU CHLOROFORME SUR LE FOIE DE SOURIS SOUMISES À UN RÉGIME PAUVRE EN PROTEINES**

**SOMMAIRE**

Une exposition unique de quarante-cinq minutes à l’halothane n’a pas causé d’altérations pathologiques démontrables du foie quand il était administré à des souris en atmosphère d’air ou d’oxygène et quel que soit leur régime, normal ou pauvre en protéines. D’une façon analogue, une augmentation des tensions de l’oxygène inhalé n’avait pas d’influence per se sur les altérations du foie. L’hépatotoxicité bien connue du chloroforme a été démontrée dans des groupes ayant un régime tant normal que pauvre en protéines. Les animaux ont moins bien toléré l’anesthésie unique au chloroforme que ceux qui ont reçu l’halothane, avec d’importantes lésions hépatiques dans les deux groupes.

**DIE AUSWIRKUNGEN VON HALOTHAN UND CHLOROFORM AUF DIE LEBER BEI MAUSEN MIT EIWEISSMANGEL**

**ZUSAMMENFASSUNG**