Comparative Toxicities of Halothane, Isoflurane, and Diethyl Ether at Subanesthetic Concentrations in Laboratory Animals


Effects of 35-day exposures to subanesthetic concentrations of halothane, isoflurane, and diethyl ether were measured in mice, rats, and guinea pigs which were in a phase of rapid body growth. Halothane produced a greater decrement in weight gain and a greater incidence of hepatic degenerative changes than isoflurane or diethyl ether despite its administration at lower anesthetic concentrations. Isoflurane results were intermediate between those of halothane and diethyl ether. No consistent injury to any organ other than the liver was found. (Key words: Anesthetics, volatile, halothane; Anesthetics, volatile, isoflurane; Anesthetics, volatile, diethyl ether; Liver, hepatotoxicity; Toxicity, hepatic.)

Modern inhalation anesthetics all have demonstrated toxicity to liver or kidney. For the most popular of these anesthetics, halothane, at least two explanations have been suggested for its hepatotoxicity. One is an allergic or sensitization phenomenon. A second, less widely accepted, view is that halothane or its metabolites cause hepatotoxicity directly. The difficulty with the latter view is that evidence of classic hepatotoxicity (pan-species, dose-related, reproducible toxic effect) is lacking.

We sought to develop a method that would test the hepatotoxic effect of metabolites of halothane. Our approach was to use a method of exposure to halothane that exaggerated the production of metabolites and gave sufficient time for them to exert a toxic effect. To do this we administered subanesthetic concentrations for prolonged periods. Our rationale for this approach was based on the demonstration by Sawyer et al. that a dose is attained beyond which the liver does not metabolize an increasing amount of the halothane presented to it. That is, increasing the concentration above that dose does not increase the amount metabolized—probably because of saturation of the enzymes responsible for metabolism. This concentration is approached approximately between 1/10 and 1/100 MAC. Thus, the amount of metabolite produced per unit time may be approximately the same at 1 MAC and 1/10 or even 1/100 MAC. If the exposure to the lower dose is prolonged greatly, the amount of metabolite produced is increased accordingly. The prolonged low-dose approach also was suggested by Chenoweth et al., who found that hepatic injury could be produced by exposure to low concentrations of halothane and methoxyflurane, but not diethyl ether. The exposures they used were 7 hours/day, 5 days/week, for 7 weeks.

If anesthetic metabolites are important in the toxicity of an inhaled anesthetic, then an anesthetic that is resistant to metabolism

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(such as isoflurane, Forane®) or is metabolized to products normally in the metabolic pool (such as diethyl ether) should be less toxic than an agent that is metabolized in significant quantities and whose metabolites are toxic (such as halothane). In the present study we tested this hypothesis by comparing the effects on body growth rates and organ histology of subanesthetic concentrations of halothane, isoflurane, and diethyl ether.

Methods

We exposed groups of 16 Sprague-Dawley rats, 16 Hartley guinea pigs and 48 ICR mice to each of the anesthetic concentrations listed in table 1. The animals were young and were in an active phase of growth. We chose guinea pigs whose weights were between 250 and 350 g; rats, 150 and 275 g; mice, 18 and 20 g. Animals were divided equally between male and female. The two sexes were caged separately. The animals were housed in a chamber in which the temperature was controlled at 22–23°C. Air was circulated by two routes, through a carbon dioxide (soda lime) absorber, and through an air conditioner. Fresh gas inflow consisted of air plus oxygen. Measured oxygen concentrations (Beckman-Pauling meter) were 21–24 per cent. Carbon dioxide levels, measured intermittently by gas chromatography, never exceeded 0.37 per cent. Oxygen was delivered via Fluomatic (halothane, isoflurane) or Pentamatic (ether) vaporizers. The anesthetic concentration was measured automatically at four-hour intervals by gas chromatography.

The chamber was entered and the animals weighed twice weekly. At this time cages were cleaned and food and water were replenished. Wire mesh traps beneath each cage allowed us to collect and weigh the total fecal output between animal weighings. The chamber also was entered on intervening days if necessary to renew food and water supplies. However, the chamber was not opened more than once daily, and never for more than two hours. The animals were exposed to a 24-hour cycle of 12 hours of light and 12 hours of darkness.

Control groups of eight rats, eight guinea pigs, and 16 mice were treated identically except for omission of the anesthetics. Traces of agent found in the control chamber always were less than 1/100 of the concentration in the experimental chamber.

In order to assess the importance of decreased food intake in development of degenerative hepatic lesions, we limited food intake in two groups of mice so that their weight gains paralleled weight gains seen at 0.005, 0.015 and 0.03 per cent halothane and 0.1 and 1.0 per cent ether. These “starved” animals were sacrificed at the same time as those exposed to the anesthetics.

Five days prior to the onset of anesthetic exposure, all animals (including control animals) were housed in the environmental chamber. Animals that failed to gain weight in this period were replaced. The anesthetic regimen was then instituted and maintained continuously for five weeks, with the following exceptions. Four male and four female mice were sacrificed between 1½ and 2 weeks. An identical group also was sacrificed between 3½ and 4 weeks. This was done to determine the rate of development of histologic lesions. In the 0.03 per cent halothane experiment, guinea pigs and mice were sacrificed after 8 and 21 days, respectively, because by this time approximately 25 per cent of each of these species had died and we feared loss of all animals would occur if we persisted. Animals that died were subject to autolysis, which prevented adequate histologic examination of tissues. In the 1 per cent ether experiment, guinea pigs were sacrificed after 20 days and mice after 20 days for the same reason. In the 0.015 per cent halothane studies, 31 per cent of mice and 38 per cent of guinea pigs died prior to 35 days. Other than these losses, occasional mice and guinea pigs in the experimental groups died, but the fractions lost did not exceed 13 per cent of mice or 31 per cent of guinea pigs in the 35-day exposure period. All rats survived

†† Trademark of Airco, Inc., Ohio Medical Products Division.
<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Anesthetic Concentration Vol Per Cent (Fraction of MAC)*</th>
<th>Mice</th>
<th>Rats</th>
<th>Guinea Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Mice</td>
<td>Control (G)</td>
<td>Experimental (G)</td>
<td>C - Ex</td>
</tr>
<tr>
<td>7 days</td>
<td></td>
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<td></td>
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<tr>
<td>Halothane</td>
<td>0.03</td>
<td>C 16</td>
<td>2.8</td>
<td>-3.2</td>
</tr>
<tr>
<td></td>
<td>(1/50 to 1/37)</td>
<td>Ex 48</td>
<td>± 0.7</td>
<td>± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>C 16</td>
<td>1.8</td>
<td>-0.7</td>
</tr>
<tr>
<td></td>
<td>(1/100 to 1/75)</td>
<td>Ex 48</td>
<td>± 0.6</td>
<td>± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>C 16</td>
<td>2.5</td>
<td>-1.5</td>
</tr>
<tr>
<td></td>
<td>(1/350 to 1/200)</td>
<td>Ex 48</td>
<td>± 0.5</td>
<td>± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.0015</td>
<td>C 16</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(1/1,000 to 1/600)</td>
<td>Ex 48</td>
<td>± 0.6</td>
<td>± 0.4</td>
</tr>
</tbody>
</table>

| Isoflurane | 0.15       | C 16          | 1.8          | -0.7  | 2.5‡          | "              | "            | 30.2  | 2.2 NS        | C 8          | 62             | 41         |
|           | (1/5 to 1/10) | Ex 48          | ± 0.4        | ± 0.4 |               | "              | "            | ± 3.9 |               | Ex 16          | ± 9             | ± 8        |
|           | 0.05       | C 32          | 2.9          | 1.0   | 1.0‡          | "              | "            | 30.8  | 1.6 NS        | C 8          | 49             | 7          |
|           | (1/16 to 1/30) | Ex 48          | ± 0.6        | ± 0.4 |               | "              | "            | ± 3.9 |               | Ex 16          | ± 19            | ± 9        |
|           | 0.015      | C 16          | 2.5          | 2.1   | 0.4 NS        | "              | "            | 36.1  | -3.7 NS       | C 8          | 58             | 42         |
|           | (1/50 to 1/100) | Ex 47          | ± 0.7        | ± 0.5 |               | "              | "            | ± 3.9 |               | Ex 16          | ± 12            | ± 11       |

<p>| Diethyl ether | 1.0       | C 32          | 2.8          | 2.8   | 0 NS          | &quot;              | &quot;            | 22.6  | 9.8†          | C 7          | 24             | -21        |
|              | (1/3)     | Ex 48          | ± 0.2        | ± 0.3 |               | &quot;              | &quot;            | ± 2.0 |               | Ex 15          | ± 20            | ± 11       |
|              | 0.1       | C 32          | 3.3          | 2.6   | 0.7 NS        | &quot;              | &quot;            | 23.9  | 6.5 NS        | C 8          | 45             | 22         |
|              | (1/30)    | Ex 48          | ± 0.3        | ± 0.3 |               | &quot;              | &quot;            | ± 3.3 |               | Ex 15          | ± 10            | ± 10       |</p>
<table>
<thead>
<tr>
<th>14 days</th>
<th>Halothane</th>
<th>0.03 (1/50 to 1/37)</th>
<th>0.015 (1/100 to 1/75)</th>
<th>0.005 (1/350 to 1/200)</th>
<th>0.0015 (1/1,000 to 1/600)</th>
<th>Isoflurane</th>
<th>0.15 (1/5 to 1/10)</th>
<th>0.05 (1/16 to 1/30)</th>
<th>0.015 (1/50 to 1/100)</th>
<th>Diethyl ether</th>
<th>1.0 (1/3)</th>
<th>0.1 (1/30)</th>
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<tr>
<td></td>
<td></td>
<td>C 16</td>
<td>Ex 37</td>
<td>C 14</td>
<td>Ex 36</td>
<td>C 16</td>
<td>Ex 45</td>
<td>C 31</td>
<td>Ex 48</td>
<td>C 32</td>
<td>Ex 48</td>
<td>C 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6 ± 0.8</td>
<td>-4.6 ± 0.3</td>
<td>4.8 ± 0.8</td>
<td>-0.8 ± 0.4</td>
<td>6.0 ± 0.7</td>
<td>Ex 48</td>
<td>5.0 ± 0.7</td>
<td>-0.7 ± 0.5</td>
<td>2.6 ± 0.7</td>
<td>Ex 48</td>
<td>5.4 ± 0.4</td>
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<tr>
<td></td>
<td></td>
<td>7.2</td>
<td>Ex 16</td>
<td>57.8 ± 3.3</td>
<td>5.6</td>
<td>Ex 16</td>
<td>5.5</td>
<td>Ex 16</td>
<td>5.0 NS</td>
<td>3.7</td>
<td>Ex 16</td>
<td>0.8 NS</td>
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<td></td>
<td></td>
<td>57.8 ± 3.3</td>
<td>-5.2 ± 0.7</td>
<td>24.4 ± 5.4</td>
<td>33.41</td>
<td>C 4</td>
<td>98 ± 6</td>
<td>Ex 12</td>
<td>8.3 NS</td>
<td>40.5 ± 4.6</td>
<td>Ex 12</td>
<td>53.8 ± 7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63.0</td>
<td>Ex 16</td>
<td></td>
<td></td>
<td>C 4</td>
<td>101 ± 9</td>
<td>Ex 16</td>
<td></td>
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<td></td>
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<td>1701</td>
<td>98 ± 23</td>
<td>781</td>
<td></td>
<td>23 ± 9</td>
<td></td>
<td></td>
<td></td>
<td>41 ± 6</td>
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<td>94 ± 6</td>
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</table>

* Fraction of MAC, first listed value pertains to mice, second to rats and guinea pigs.
† C - Ex = control minus experimental animal weight change.
\$ P < 0.001.
\$ P < 0.005.
\$ P < 0.01.
\$ P < 0.05.
NS = not significant.
<table>
<thead>
<tr>
<th>Aesthetic</th>
<th>Concentration (Vol Per Cent, Fraction of MAC*)</th>
<th>Number of Mice</th>
<th>Number of Rats</th>
<th>Number of Guinea Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Control (C)</td>
<td>Experimental (Ex)</td>
<td>Control (C)</td>
</tr>
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</tr>
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<td>Halothane</td>
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<td></td>
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<tr>
<td>0.03</td>
<td>(1/50 to 1/37)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.015</td>
<td>(1/100 to 1/75)</td>
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<tr>
<td>0.005</td>
<td>(1/350 to 1/200)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.0015</td>
<td>(1/1,000 to 1/600)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>(1/5 to 1/10)</td>
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<tr>
<td>0.05</td>
<td>(1/16 to 1/30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.015</td>
<td>(1/50 to 1/100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>(1/3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.1</td>
<td>(1/30)</td>
<td></td>
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<td></td>
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</tbody>
</table>

* Fraction of MAC, first listed value pertains to mice, second to rats and guinea pigs.

† Control minus experimental animal weight change.

NS = not significant.
all 35-day exposures. In the 0.0015 per cent halothane study, four experimental and two control female guinea pigs were deleted because they were pregnant on receipt from the supplier. In other control groups, as many as two guinea pigs and two mice sometimes failed to survive the entire 35-day exposure period.

All animals were killed by carbon dioxide inhalation at 35 days (earlier in the case of mice and guinea pigs exposed to 0.03 per cent halothane and 1 per cent ether). Heart, lungs, liver, kidney and spleen were weighed and fixed in 10 per cent formalin. Pieces of skeletal muscle, jejunum, proximal femur, and brain were also preserved in 10 per cent formalin. All liver specimens were examined microscopically. Microscopic examinations were performed by Dr. David Slauson, D.V.M., M.A., Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico. His examination was “blind” in that he was not aware of the anesthetic regimen to which any group was subjected.

All kidney specimens from isoflurane-treated animals were examined microscopically. Every organ was examined in a limited number of animals in each experimental and control group. Two animals in each experimental group and one animal in each control group were subjected to such examination.

Blood was obtained from rats exposed to 1 per cent ether for 35 days and from rats exposed to 0.05 per cent isoflurane for 35 days. Hematocrits and erythrocyte, leukocyte, and differential counts were measured.

Mean and standard error were computed for weight changes. Weight gains of control and experimental animals were compared at 7, 14, and 35 days by a t test for unpaired data. The control groups for each species were compared by an analysis of variance. These tests revealed no differences in rat control groups, which therefore were pooled. However, the control groups of guinea pigs and mice showed significant weight differences. Therefore, each experimental group was compared with its own control group.

Livers were examined for the presence or absence of degenerative lesions, which included granular, vacuolar degeneration, zonal centrilobular lipidosis, focal lipidosis, and focal necrosis. These constituted 85 per cent of the degenerative lesions seen. We considered a liver to be abnormal if any degenerative lesion was present. No weight was given to the appearance of multiple lesions. A number of the controls had abnormal livers by these criteria. The incidence of abnormalities in the total control group for each species was subtracted from the incidence in each experimental group. The residual percentage was then divided by the difference between 100 per cent and the percentage incidence in control groups. This gave a fractional increase in degenerative lesions in experimental groups. These incidences were plotted against dose and subjected to probit analysis.

Our definition of “degenerative changes” excluded other abnormalities that we detected occasionally in both control and experimental animals. These included focal granulomatous or supplicative inflammation, vascular abnormalities such as passive congestion, pigment deposition, extramedullary hemopoiesis and ductal hyperplasia.

All anesthetic concentrations are expressed as fraction of MAC. For halothane, MAC in rats was determined in our laboratory to be 1.11 per cent. This value was assumed to apply to guinea pigs. We assumed MAC for mice to be approximately equivalent to the dose that abolishes the righting reflex. MAC for isoflurane in rats is 1.38 per cent. This value was assumed to apply in guinea pigs. MAC for isoflurane in guinea pigs was 0.9 per cent.11 We assumed that MAC for ether for all three species was identical to ether MAC in dogs, 3.0 per cent.6

Results

Halothane produced a dose-related detrimental effect on weight gain in all species (table 1, figs. 1 and 2). In contrast, diethyl ether had a negligible effect on rat or mouse weight gain and a detrimental effect in guinea pigs at the 1 per cent dose only. One per cent ether was lethal to guinea pigs and mice despite its relatively small effect on growth. The isoflurane doses and their effects

11 Personal communication, David W. Kent, M.D., Department of Anesthesia, University of California, San Francisco.
were intermediate between halothane and ether results. A small detrimental effect occurred at 0.15 per cent isoflurane in guinea pigs and a significant effect on weight gain occurred in mice, primarily at 0.15 and 0.05 per cent.

In general, changes of organ weights tended to parallel changes in body weights, with the exception of the livers. Analysis of variance (halothane) or t test for unpaired data (ether) suggested that the ratio of liver to total body weight was smallest with lowest anesthetic doses. This effect was particularly striking with 1 per cent ether in male mice, where the control livers weighed 2.95 ± 0.09 g and the experimental livers weighed 5.19 ± 0.17 g after 20 days. The liver/body weight ratios were 0.087 ± 0.002 and 0.162 ± 0.004, respectively (table 2). No significant difference was found with isoflurane.

Fig. 1. Body weights of rats before and 14 and 35 days after exposures to four halothane concentrations. Control rats are combined into a single group.

Fig. 2. Body weight changes in rats in relation to type and dose of anesthetic drug and duration of exposure to the anesthetic agent. Weight change of control animals has been subtracted from that of the experimental animals. A negative value indicates greater weight loss or smaller weight gain in the experimental group compared with control.
Table 2. Comparisons of Liver Weights and Liver-Body Weight Ratios of Control Mice (C) and Mice Exposed to Diethyl Ether (Ex)

<table>
<thead>
<tr>
<th>Ether Concentration, Vol Per Cent (Fraction of MAC)</th>
<th>LIVER WEIGHT (g)</th>
<th>Liver/Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>C     Ex</td>
<td>C     Ex</td>
</tr>
<tr>
<td>1.0 (1/3)</td>
<td>2.95⁺ 5.19ab</td>
<td>2.28 3.62ab</td>
</tr>
<tr>
<td>±.09 ±.17</td>
<td>±.07 ±.10</td>
<td>±.002 ±.004</td>
</tr>
<tr>
<td>0.1 (1/30)</td>
<td>2.52 3.17a</td>
<td>2.20 2.13</td>
</tr>
<tr>
<td>±.13 ±.13</td>
<td>±.08 ±.08</td>
<td>±.002 ±.002</td>
</tr>
</tbody>
</table>

⁺ Comparisons of experimental and control results for each sex and concentration, experimental > control, P < 0.005.  
ab Comparisons of experimental animals only, 1 per cent group > 0.1 per cent group, P < 0.001.  
⁺⁺ Comparisons of control animals only, 1 per cent > 0.1 per cent group, P < 0.05.

There was no difference in blood morphology between control animals and experimental animals that received 1.0 per cent ether or 0.05 per cent isoflurane. Organs other than liver did not show any abnormality in control or experimental groups. Similarly, livers from animals exposed to isoflurane and ether manifested no or small increases in lesions compared with their control peers (table 3). Livers from halothane-treated animals developed degenerative lesions, which increased in frequency of occurrence with increasing dose (fig. 3). Probit analysis suggested that a 50 per cent incidence of lesions would occur at 0.014 (0.009 to 0.022, 95 confidence limits) per cent halothane in mice (1/60 MAC) and at 0.010 (0.005 to 0.017) per cent in rats (1/100 MAC). Distribution of the guinea pig data precluded a meaningful probit analysis. Not only was the incidence of affected animals related to halothane dose, but the incidence of lesions in any animal was greater at higher doses. The incidences of degenerative hepatic lesions in experimental mice sacrificed after 1½ and after 3½ weeks of exposure to halothane were similar.

Discussion

These data demonstrate that halothane is a hepatotoxin in the classic sense. The effect it produced was dose-related, reproducible, and present in all rodent species we tested. We found differences in susceptibility to hepatic injury among these species. Guinea pigs were most susceptible; rats were most resistant. These findings accord with data presented by others. Linde,⁷ for example, found no hepatic damage in rats exposed to 1/100 MAC halothane, whereas Hughes⁸ demonstrated hepatic necrosis in guinea pigs exposed repeatedly to 1 per cent halothane.

Why do we impute an anesthetic metabolite as the cause of hepatic injury? The finding that significant injury occurred with prolonged exposure at a dose far smaller than that used by Chenoveth; the innocuousness of ether; and the relatively small effect of isoflurane, an agent that is metabolized to a far lesser extent than halothane, are suggestive. No other interpretation seems to explain these findings. For instance, if toxicity resulted from some inherent property of anesthesia per se, we should have seen hepatic injury with the higher doses of ether or isoflurane. Instead, we saw no hepatic injury at isoflurane doses (in MAC multiples) four to ten times as great, or ether doses six to 30 times as great, as the halothane dose that produced a 50 per cent incidence of hepatic lesions. The final incrimination of halothane metabolism as the cause of hepatic injury awaits the identification of a metabolite capable of inflicting such injury.

Further evidence of the importance of metabolism to hepatic injury would be the demonstration that increased metabolism increased the incidence and/or severity of injury. Studies with one inducer of microsomal enzymes, phenobarbital, have produced inconsistent findings. Almersjö⁹ could
Table 3. Degenerative Hepatic Lesions* in Control (C) and Experimental (Ex) Animals

<table>
<thead>
<tr>
<th>Anesthetic Concentration, Vol Per Cent (Fraction of MAC)</th>
<th>Mice</th>
<th></th>
<th>Rats</th>
<th></th>
<th>Guinea Pigs</th>
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<tr>
<td></td>
<td>C</td>
<td>Ex</td>
<td>C</td>
<td>Ex</td>
<td>C</td>
</tr>
<tr>
<td>Halothane</td>
<td>3/77</td>
<td>35/35</td>
<td>7/32</td>
<td>16/16</td>
<td>6/25</td>
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<td>0.015 (1/100 to 1/75)</td>
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<td>0.005 (1/350 to 1/200)</td>
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<tr>
<td>Isoflurane</td>
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<td>8/31</td>
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<td>6/21</td>
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<td>0.1 (1/30)</td>
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* Numerator = number of animals with degenerative lesions; denominator = number of specimens available for histologic analysis. The control animals were considered as a single group.
† Fraction of MAC: first number pertains to mice, second to rats and guinea pigs.

not demonstrate hepatic injury in phenobarbital-treated rats after a single exposure to 0.5 to 1.5 per cent halothane for 150 minutes. On the other hand, Stenger et al. demonstrated that intraperitoneal administration of halothane produced multifocal necrosis in phenobarbital-pretreated rats but not in animals that had not received phenobarbital. Reynolds et al. found that rats pre-treated with phenobarbital and anesthetized for 5 hours with 0.85 per cent halothane manifested hepatic necrosis that was largely but not exclusively centrilobular and was predominantly in a subcapsular location in posterior portions of the liver. Saline-pretreated rats were not injured by an identical anesthetic experience.

Most occurrences of toxicity in patients have developed after repeated exposures. Repetition might enhance metabolism of the agent. Although our experiments demonstrated hepatotoxicity in animals, clinical experience indicates that only a small proportion of patients will be adversely affected by halothane. Such patients may be particularly susceptible to injury by metabolites. A greater susceptibility to the noxious effects of the metabolites may be conferred by hepatic hypoxia (induced by either hypoxemia or a reduction of hepatic blood flow) or by starvation and a reduction in hepatic antioxidants. Additionally, an occasional patient, by some quirk of metabolism, may generate more, or different, more noxious, metabolites.

Are our results in laboratory animals applicable to man undergoing anesthesia? Clearly, laboratory animals differ from man, and our method of anesthetic administration was far removed from that used in clinical practice. Another inhaled anesthetic, fluroxene, is exceedingly toxic to mice, rats, guinea pigs, rabbits, cats, and dogs, but not to man. This animal–human difference apparently results from differing patterns of anesthetic metabolism in animals and man. Such differences in metabolism also apply to halothane. Prolonged administration at low doses may represent an unusual test of an agent's toxicity in that exposure to metabolites, in terms of both amount and duration (i.e., total dose) may be greater than with clinical use. The low-dose exposure may serve to enhance toxicity in a manner that is useful in comparative studies of drugs but yet not quantitatively transferrable to clinical conditions.

Although we believe our results are related to clinical halothane toxicity, they may provide only a partial answer. Hepatic toxicity in man may be a dual phenomenon. Hepatic injury may be produced by metabolites or by sensitization, or by some combination of the two. Sensitization may be produced to a metabolite–protein complex that forms the
Fig. 3. Incidences of hepatic degenerative lesions in mice, rats, and guinea pigs after 35-day exposures to various doses of halothane, isoflurane and diethyl ether. The percentage of all control animals with lesions has been subtracted from the percentage of experimental animals showing similar lesions. A positive value indicates a greater incidence of lesions in experimental animals.

A patient capable of metabolizing more of the delivered dose of halothane thus would be more susceptible to either sensitizing or direct toxic effects. A patient capable of metabolizing less of the delivered dose of halothane would, therefore, be more susceptible to sensitizing or direct toxic effects. How can our studies of low concentrations in animals apply to man exposed only to high concentrations for short periods? The exposure of man may more closely approximate our experiments than is at first apparent. As shown by Cohen and by Topham in laboratory animals, halothane persists in the body for long periods in the course of recovery from anesthesia. Halothane or its metabolites can be recovered from exhaled gases for at least six days after anesthesia in man and can be measured in urine and feces for even longer, presumably due to storage of halothane in fat reservoirs. Thus, metabolism following anesthesia is a function of uptake of agent which, in turn, is a function of anesthetic concentration and duration of exposure.

Perhaps some extent of hepatic injury following halothane anesthesia in man is not as rare as the incidence of massive hepatic necrosis would suggest. Thompson and Greifenstein found greater SGPT elevation following halothane and methoxyflurane compared with spinal anesthesia. We have demonstrated greater and more prolonged elevations of sulfobromophthalein retention in volunteers subjected to halothane, fluroxene, or ether than in volunteers anesthetized for similar periods with isoflurane. Ackman et al. found abnormal SGPT elevation more frequently after halothane or methoxyflurane than after anesthesia with non-halogenated compounds. Such studies would indicate that a mild hepatic injury is far from uncommon following halothane anesthesia.

The “classic” hepatotoxins, carbon tetrachloride and chloroform, characteristically cause centrilobular hepatic injury, perhaps as a result of the greater concentration of drug-metabolizing enzymes in centrilobular hepatocytes. We have no explanation for the more diffuse nature of the injury we saw. However, our findings are not unique. Hepatic necrosis after halothane also was of a patchy, more diffuse nature in studies reported by Hughes et al. and Stenger et al. Confinement to a centrilobular locus was not seen by Reynolds et al. in phenobarbital-treated rats.

It is not clear what role nutritional changes played in our results. It is known that hepatic injury by methoxyflurane is enhanced by starvation. However, our liver-injured animals were not starved, and indeed their fecal
output equaled that of their control peers. Furthermore, in our study, starved but non-
anesthetized animals did not show hepatic injury. The liver/body weight ratios of
starved animals were similar to control ratios. We are unaware of the effects of starvation on
anesthetic metabolism or of the liability to injury from subanesthetic concentrations of
drugs.

Although no hepatic injury was seen with
the higher dose of ether, this dose was lethal
to mice and guinea pigs but not rats, for
unknown reasons. The mortality was unre-
lated to changes in blood or the appearance
of histologic changes in any tissue examined,
including bone marrow. These animals did
manifest gross hepatic enlargement, similar
to that found by Chenoweth. Perhaps ether
or its metabolism to ethyl alcohol stimulated
growth of the liver, but how this might be
related to ether lethality is unclear.

Do these results imply a hazard to the
anesthetist who breathes low concentrations
of halothane over long periods? It appears
from our data that liver injury may occur at
a concentration of between 0.0015 and 0.005
per cent halothane when exposure is con-
tinuous. These concentrations exceed the
0.0001 to 0.001 per cent found in the area of
the anesthesia machine. Whether an occult
effect is produced by the lower doses en-
countered by operating room personnel
remains to be determined. Prudence suggests
that inhalation of these low concentrations
should be avoided if possible through the use
of anesthetic scavenging systems.

References

1. Dykes MJM, Gilbert JP, Schur PH, et al:
Halothane and the liver: A review of the
epidemiologic, immunologic and metabolic
aspects of the relationship. Can J Surg
15:1–22, 1972

2. Sawyer DC, Eger EI II, Bahlman SH, et al:
Concentration dependence of hepatic
halothane metabolism. ANESTHESIOLOGY
34:230–235, 1971

3. Chenoweth MB, Leong BKJ, Sparschul GL, et
al: Toxicities of methoxyflurane, halothane
and diethyl ether in laboratory animals on
repeated inhalation at subanesthetic concen-
trations, Cellular Biology and Toxicity of
Anesthetics. Edited by BR Fink. Baltimore,
Williams and Wilkins, 1972, pp 273–285

4. White PF, Johnston RJ, Eger EI II: Determina-
tion of anesthetic requirement in rats.
ANESTHESIOLOGY 40:52–57, 1974

5. Paton WDM, Speden RN: Analysis of the
kinetics of anesthesia in mice. Br J Phar-
macol Chemother 25:88–103, 1965

Equivalent alveolar concentrations of
methoxyflurane, halothane, diethyl ether,
fluoroxene, cyclopropane, xenon and nitrous
oxide in the dog. ANESTHESIOLOGY
26:771–777, 1965

7. Linde HW, Bruce DL: Effects of chronic
exposure of rats to traces of halothane.
Proceedings of the 4th World Congress
of Anesthesiologists. Edited by TB Boulton, R
Bryce-Smith, MK Sykes et al. Amsterdam,
Excerpta Medica Foundation, 1970, pp
923–926

8. Hughes HC Jr, Lang CM: Hepatic necrosis
produced by repeated administration of
halothane to guinea pigs. ANESTHESIOLOGY
36:466–471, 1972

9. Almersjo O: Influence of halothane anesthesia
on the rat liver with stimulated drug
metabolism. Acta Chir Scand suppl 416, pp
57–60, 1971

10. Stenger RJ, Johnson FA: Effects of phenobar-
bital pretreatment on the response of rat
liver to halothane administration. Proc Soc

11. Reynolds ES, Moslen XT: Liver injury follow-
ing halothane anesthesia in phenobarbital
pretreated rats. Biochem Pharmacol
23:189–193, 1974

12. Van Dyke RA, Wood CL: Binding of radioac-
tivity from 14C-labeled halothane in isolated
perfused rat livers. ANESTHESIOLOGY
35:348–353, 1971

The toxicity of fluoroxene in animals and
man. ANESTHESIOLOGY 38:313–319, 1973

Anesthesia LXXIV: Biotransformation of
fluoroxene. I. Metabolism in mice and dogs in
vivo. Biochem Pharmacol 16:1237–1248,
1967

15. Cohen EN: Metabolism of the volatile
anesthetics. ANESTHESIOLOGY 35:193–202,
1971

mental immunity to a metabolite of halothane and
fluoroxene: Cutaneous delayed
hypersensitivity. ANESTHESIOLOGY 40:385–
390, 1974

17. Cascorbi HF, Blake DA, Helrich M: Differe-
ces in the biotransformation of halothane

18. Cohen EN: Metabolism of halothane-214C
in the mouse. ANESTHESIOLOGY 35:348–353,
1971

19. Topham JC, Longshaw S: Studies with
halothane: I. The distribution and excretion
of halothane metabolites in animals. Anesthesiology 37:311–323, 1972


Neonatology

HYPERNATREMIA AND INTRACRANIAL HEMORRHAGE The authors have reviewed the incidences of hypernatremia and intracranial hemorrhage in newborns admitted to their unit in a two-year period. In the second year of the study the total intake of sodium bicarbonate was kept at a minimum and the total intake of sodium less than 8 mEq/kg/day. The occurrence of hypernatremia decreased from 8.8 to 0.6 per cent and the incidence of intracranial hemorrhage declined from 13.4 to 2.6 per cent. Hypernatremia (or hyperosmolality) reduces the intracranial pressure by decreasing the water content of the brain, thus increasing the traction of the small vessels, leading to their rupture. The therapeutic value of sodium bicarbonate is disputed, as the most important variable for pulmonary vascular resistance is P CO2, not H+ concentration. Simms, M. A., and others: Hypernatremia and Intracranial Hemorrhage in neonates. N Engl J Med 291:6–10, 1974.) ABSTRACTER’S COMMENT: This may be true for a situation in the “medical” newborn nursery. However, the surgical neonate may require relatively large amounts of sodium for maintenance, especially if the condition is associated with electrolyte loss, as in a ruptured omphalolec, meconium ileus, or intestinal volvulus.