Uptake and Elimination of Methoxyflurane as Influenced by Enzyme Induction in the Rat

M. Lawrence Berman, M.D.,* Harry J. Lowe, M.D.,†
Julius Bochantin, B.S., Karl Hagler, B.S.

Pretreatment of rats with an enzyme-inducing dose of phenobarbital (75 mg/kg/day for three days) resulted in the following responses relative to controls: 1) the rate of whole-body uptake of methoxyflurane (ml vapor/kg/hour) as measured in a closed system was increased by 36 per cent and the rate of elimination was decreased by 21 per cent; 2) metabolism of methoxyflurane as determined by the content of inorganic fluoride in livers and kidneys was markedly increased. These effects of phenobarbital were partially blocked by an inhibitor of microsomal enzymic activity. Blood concentrations of methoxyflurane were significantly less than control values during administration of and recovery from anesthesia. Treated rats took longer to be anesthetized than controls. These results suggest that by accelerating the rate at which methoxyflurane is metabolized enzyme induction influences the amounts of anesthetic taken up and eliminated. (Key words: Enzyme induction; Microsomes; Inhalational anesthetics; Uptake; Metabolism; Elimination; Methoxyflurane; Phenobarbital.)

Van Dyke1 has shown that phenobarbital induces the liver microsomal enzyme systems which cleave the ether linkage in methoxyflurane (MOF) and dechlorinates MOF and halothane. Previous studies in this laboratory2,3 have demonstrated that induction of enzymes by chronic exposure of rats to subanesthetic concentrations of the vapors of MOF or by intraperitoneal injections of several chemicals used as preservatives in volatile anesthetics permitted survival of rats at MOF concentrations lethal to nontreated animals.

Inhibition of the microsomal drug-metabolizing enzyme system reduced the survival rate during exposure to MOF. These findings suggested that enzyme induction accelerated the rate at which MOF is metabolized and enhanced tolerance to the anesthetic.

In the present investigation the uptake and elimination of MOF were determined in phenobarbital-treated and control rats. We observed that phenobarbital treatment significantly increased uptake and decreased elimination of MOF.

Methods

Male Sprague-Dawley Charles River Random Strain littermate rats were used. They were placed in plastic cages 45 × 23 × 13 cm containing a non-chemically-treated cellulose bedding (San-i-cell) which does not interfere with drug-metabolizing enzymes.4 The animals were housed in a room maintained at 24–26 °C with cycling of fresh air every 5 minutes and 12 hours of light per day. The rats were fed insecticide-free Purina Rat Chow5 and water ad lib. Care was also taken to prevent the rats' exposure to other materials known to affect drug-metabolizing enzymes.6,7 such as insecticides and cedar wood shavings. Forty-eight hours after their arrival at our animal quarters, the littermates were divided into a treatment and a control group and kept in separate, but identical, cages as described.

Phenobarbital was used as the enzyme-inducing agent. Sodium phenobarbital was dissolved in 0.9 per cent sodium chloride solution. Rats weighing 50 ± 5 g were given the drug ip at a dose of 75 mg/kg/day (1 ml/kg) for three consecutive days at 9:00 A.M. each day. Controls (littermates) received the vehicle, 1 ml/kg, for the same period. The effectiveness of this phenobarbital dose schedule in causing enzyme induction was evaluated by comparing enzymic activity, quantity

* Assistant Professor, Department of Anesthesia, Northwestern University.
† Professor and Chairman, Department of Anesthesiology, The University of Chicago.

Received from the Department of Anesthesia, Northwestern University Medical School and the Department of Anesthesiology, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60611. Accepted for publication November 15, 1972. Supported in part by USPHS, National Institutes of Health grant GM 15420-04.

352
of protein, and cytochrome P-450 content in microsomes prepared from livers of rats treated with phenobarbital with control values. To test for inhibition of liver microsomal enzymatic activity, SKF 525-A (beta-diethylamino-phenylpropylacetate HCl), 8 50 mg/kg, was given to phenobarbital-treated and control rats ip 40 minutes prior to preparing microsomes. Microsomes were prepared from livers of rats 24 hours after the final dose of either phenobarbital or the vehicle, according to the method of Berman. 5 The ability of microsomes to dechlorinate 36Cl-1,1,2-trichloroethane enzymatically was determined by the amount of inorganic 36Cl formed, using the method described by Van Dyke. 9 Microsomal protein was measured by a modification 10 of the method of Lowry. 11 Cytochrome P-450 was determined spectrophotometrically according to the method of Omura, 12 using a Beckman Model DU spectrophotometer.

Three experiments on the influence of pre-treatment with phenobarbital were performed to determine: 1) the quantities of MOF vapor taken up and eliminated during induction of and recovery from anesthesia; 2) the extent of metabolism; 3) blood concentrations of MOF during uptake and elimination.

Quantities of MOF Vapor Taken Up and Eliminated

Twenty-four hours after the third dose of phenobarbital or saline solution rats were exposed to 0.5 per cent methoxyflurane in oxygen on separate days, but at similar times, to determine uptake and elimination. Measurement of uptake and elimination was accomplished by placing four rats, control or treated, in a temperature-controlled chamber of known volume containing KOH solution to absorb CO2.12 Gas mixing in the chamber was facilitated by use of a magnetic stirrer. The chamber was rapidly flushed to the desired anesthetic concentration and sealed. Every 6 seconds 0.01 ml of gas was removed automatically from the chamber and fed into a chromatograph for analysis (analysis time less than 2 seconds). The rate of uptake (ml vapor/min/kg rat) of anesthetic was calculated from the linear slope of decreasing peak heights (0.5 to 0.475 per cent MOF) and the volume of the chamber minus the weight of the animals. The chamber was flushed every 2 to 3 minutes with anesthetic vapor and the rate of uptake redetermined. At the end of 60 minutes the chamber was flushed every 2 to 3 minutes with 100 per cent oxygen, resealed, and the rate of anesthetic elimination was calculated from the linear slope of increasing peak heights measured at an attenuation of 1/128 (0 to 0.004 per cent MOF).

Extent of Metabolism of MOF Following Uptake

An hour after anesthesia with MOF, the rats were killed by decapitation. The livers and kidneys were thoroughly perfused with isotonic KCl solution and 25 per cent homogenates of these organs were prepared. The concentration of inorganic fluoride, which results from cleavage of the ether linkage of MOF (o-dealkylation) by liver microsomal enzymes, 14 was determined in liver and kidney homogenates prepared from treated and control rats using the fluoride-ion electrode according to the method of Holaday. 15

Blood Concentrations of MOF During Administration of and Recovery from Anesthesia

A phenobarbital-treated rat or a control rat was placed in a cylindrical glass chamber corked at both ends. Through one end, 0.5 per cent MOF in oxygen entered. Through the other end, which served as exit for the gas, the animal's tail protruded. The tip of the tail was cut and blood collected in capillary tubes and immediately analyzed for concentration of MOF by gas chromatography. 13 Blood samples were obtained every 3 to 5 minutes during one hour of anesthesia and during one hour of recovery (with the animal breathing 100 per cent oxygen). Induction of anesthesia was indicated by absence of spontaneous movements and failure to respond to an auditory stimulus (percussion of the glass chamber with a steel mallet) by movement or an alerting reaction.

Results

Phenobarbital treatment caused the rats to decrease their spontaneous activity and move with a staggering gait. These behavioral effects were observed following injection of the
### Table 1. Effects of the Phenobarbital Dose Schedule on Liver Microsomal Dechlorination, Microsomal Protein, and Cytochrome P-450 Content

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Inorganic Chloride-30 Released, cpm/mg Wet Weight Microsomal Protein (Mean ± SD)</th>
<th>Microsomal Protein, mg/g Wet Weight of Liver (Mean ± SD)</th>
<th>Cytochrome P-450, Nanomoles/mg Wet Weight Microsomal Protein (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenobarbital Control</td>
<td>3,908 ± 576†</td>
<td>31.3 ± 1.3†</td>
<td>1.8 ± 0.3†</td>
</tr>
<tr>
<td>Phenobarbital and SKF 525-A, 50 mg/kg</td>
<td>2,017 ± 766†</td>
<td>30.2 ± 1.4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Control and SKF 525-A, 50 mg/kg</td>
<td>274 ± 30†</td>
<td>22.3 ± 1.6</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

* Each value represents the mean for six rats.
† P < 0.001 compared with control.
‡ P < 0.01 compared with phenobarbital pretreatment.

### Table 2. Uptake and Elimination of Methoxyflurane

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uptake</th>
<th>Elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml Vapor/kg/Hour (Mean ± SD)</td>
<td>Per Cent of Control</td>
</tr>
<tr>
<td>Control</td>
<td>77.9 ± 5.7</td>
<td>—</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>108 ± 11.2†</td>
<td>136</td>
</tr>
<tr>
<td>SKF 525-A†</td>
<td>75.9 ± 2.0</td>
<td>97</td>
</tr>
<tr>
<td>Phenobarbital and SKF 525-A†</td>
<td>80.8 ± 5.7§</td>
<td>103</td>
</tr>
</tbody>
</table>

* The number of rats in each group was 24.
† Administered ip 50 mg/kg 75 minutes prior to exposure of the rats to MOF anesthesia.
‡ P < 0.005 compared with control.
§ P < 0.01 compared with phenobarbital.
¶ P < 0.05 compared with control.

### Table 3. Metabolism of Methoxyflurane (MOF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Methoxyflurane Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, µg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2,784</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>3,786</td>
</tr>
<tr>
<td>SKF 525-A</td>
<td>2,711</td>
</tr>
<tr>
<td>Phenobarbital and SKF 525-A†</td>
<td>2,878</td>
</tr>
</tbody>
</table>

* Each value represents the mean for 12 rats.
† Ratio of inorganic fluoride to total fluoride in MOF × 100.
‡ P < 0.001 compared with control.
drug and lasted about four hours. The treated animals showed similar spontaneous activity and weight gain, as did the control group 24 hours after their final dose of phenobarbital.

The effectiveness of the phenobarbital dose schedule in causing enzyme induction of the rat liver microsomal enzyme system that dechlorinates MOF is shown in table 1. Inhibition of this enzyme system by SKF 525-A was significantly less in rats pretreated with phenobarbital than in controls.

**Quantities of MOF Vapor Taken Up and Eliminated**

Significantly more MOF vapor (36 per cent more) was taken up by the phenobarbital-treated group than by the control group (table 2). SKF 525-A blocked the increase in uptake in treated rats, but did not affect uptake in control rats. During the 60 minutes after termination of MOF anesthesia, phenobarbital-treated rats eliminated significantly less (21 per cent) anesthetic vapor than did controls. Phenobarbital-treated rats given SKF 525-A eliminated about as much anesthetic vapor as did controls.

**Extent of Metabolism of MOF Following Uptake**

Table 3 shows the concentrations of inorganic fluoride in livers and kidneys of phenobarbital-treated and control rats 60 minutes after uptake of MOF. The total uptake of MOF in μM/kg was calculated from ml of vapor/kg/hour taken up (table 2). Metabolism of MOF was markedly enhanced in phenobarbital-treated rats, as demonstrated by the increases in inorganic fluoride in their livers and kidneys (sixfold increase compared with controls). The increased inorganic fluoride in these organs accounted for 3.6 to 4.2 per cent of the additional fluoride taken up as MOF by treated rats. The effect of SKF 525-A in inhibiting the metabolism of MOF was greater in controls than in phenobarbital-treated rats.

**Blood Concentrations of MOF during Administration of and Recovery from Anesthesia**

During uptake, blood levels of MOF in phenobarbital-treated rats were significantly lower than those in controls (fig. 1). Treated rats took longer to be anesthetized than the controls, but the treated group was anesthetized at about the same blood level as the control group. Phenobarbital-treated rats took less time to recover from anesthesia than did controls, although the difference between blood levels in treated rats and controls during the latter half of the 60-minute elimination period was not significant.

**Discussion**

Phenobarbital was used as the enzyme-inducing agent because it had been reported that this drug administered to the rat induces the liver microsomal enzyme system that dechlorinates MOF. As shown in table 1, we demonstrated a marked increase in dechlorinase activity concomitant with evidence for enzyme induction following pretreatment with phenobarbital. In contrast to the findings of Van Dyke, who reported that SKF 525-A had no inhibitory effect on dechlorinase activity, we observed a significant inhibition of MOF uptake and inorganic fluoride production with this compound. However, the discrepancy between the results of that study and ours may be explained by the different doses of SKF 525-A and times of administration to rats prior to removing their livers. SKF 525-A had a greater inhibitory effect on enzymic activity in the control rats compared with the treated group (tables 1 and 3). Although our data do not explain why this is so, it is possible that SKF 525-A was present in insufficient concentration to inhibit all of the enzyme present in phenobarbital-treated rats.

Our results indicate that metabolism of MOF markedly affects its uptake and elimination (table 2). Evidence supporting this concept includes: 1) the finding of significantly more inorganic fluoride in livers and kidneys of rats pretreated with phenobarbital compared with controls; 2) decreased uptake and increased elimination of MOF in phenobarbital-treated rats given SKF 525-A compared with phenobarbital-treated rats (table 1); 3) the decrease in inorganic fluoride in the livers and kidneys of rats treated with phenobarbital and SKF 525-A (table 3).
The increased level of inorganic fluoride (table 3) in the livers of treated rats sacrificed one hour after exposure to a constant concentration of anesthetic (i.e., before recovery) accounted for 3.6 per cent of the additional uptake of fluoride as MOF. (Additional whole-body uptake of fluoride was $871 - 640 = 231 \mu M/kg$; that found in the liver was $9.9 - 1.6 = 8.3 \mu M/kg$, $8.3/231 = 3.6$ per cent.) If the ratio of the metabolites of MOF, inorganic and organic fluoride, formed in rats is similar to that in man, then the amount of organic fluoride formed in induced rats would account for another 21.6 per cent ($6 \times 3.6$ per cent) of the observed 36 per cent increase in MOF uptake (table 2). Furthermore, the amount of inorganic fluoride found in the liver at the end of one hour of anesthesia may be a low concentration if it is assumed that biodegradation of MOF begins immediately after the onset of exposure to MOF, as has been reported to occur in man, and the products of metabolism are rapidly distributed to other organs and excreted in the urine. The increased concentration of inorganic fluoride in kidneys from treated rats may have been a reflection of enhanced o-dealkylation by the kidney and/or concentration of fluoride in the kidneys for excretion in the urine.

Blood levels of MOF in phenobarbital-treated rats were consistently lower than control values during uptake and elimination (fig. 1). If the difference between blood levels were due entirely to a difference between rates of metabolism, we would anticipate a continuous divergence with time of the concentrations of anesthetic in treated rats and controls. However, the blood levels were almost parallel during both uptake and elimination (fig. 1), suggesting that factors other than drug metabolism contributed to increased uptake and decreased elimination of MOF. Recent preliminary experiments in our laboratories indicate that pretreatment of rats with an enzyme-inducing dose of phenobarbital markedly increases the solubility of MOF in
several organs, including the liver. This increase in solubility could be accounted for by an increase in the total lipid in the organs. Should these preliminary findings be confirmed, it would suggest that phenobarbital, and perhaps other enzyme-inducing drugs, alter uptake and elimination of volatile anesthetics not only by enhancing their rates of biodegradation but also by altering their partition coefficients in several organs. Experiments in our laboratories designed to account for the distribution of the anesthetic and its metabolites should distinguish between metabolism and alteration in tissue composition and perfusion. From such studies it should be possible to understand more precisely how an enzyme-inducing agent increases whole-body uptake of a volatile anesthetic.

We can only speculate on the clinical significance of this study. It seems unlikely that enzyme-inducing drugs could significantly affect the conduct of clinical anesthesia; inhalation anesthetics are usually given continuously today, so that fresh gases are constantly supplied to the patient. We are attempting to determine the dose requirements of MOP and fluoride production in patients receiving drugs known to induce liver microsomal drug-metabolizing enzymes. If these drugs increase the uptake of inhalational anesthetics in man, then there is a suggestion of accelerated metabolism of the anesthetic. Since the levels of anesthetic metabolites or their intermediates are believed to be responsible for the toxicity of halogenated inhalational agents,16 enzyme-inducing drugs may enhance the toxic potential of these agents.

Enzyme induction in man usually occurs after repeated doses of the inducing agent for several days. Although there are data demonstrating that phenobarbital administered twice within 24 hours to man stimulates hepatic microsomal hydroxylase activity,17 serum fluoride levels following MOP anesthesia in patients given single doses of barbiturates preoperatively have not been significantly different from those in unmedicated patients. It seems unlikely that one dose of a short-acting barbiturate administered preoperatively would significantly stimulate the liver microsomal enzyme system.

References