Comparative Toxicity of Halothane, Isoflurane, Hypoxia, and Phenobarbital Induction in Monolayer Cultures of Rat Hepatocytes

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Halothane, phenobarbital induction, and halothane anesthesia have been implicated in the pathogenesis of hepatotoxicity in the rat model. However, a controversy exists over the role of halothane in liver injury; does it act by reducing hepatic blood flow, thereby inducing hypoxia, or do its metabolites initiate the injury? These variables are difficult to separate during in vivo halothane exposure. In the present experiments, effects of halothane on hepatic perfusion were eliminated by exposing confluent monolayers of hepatocytes isolated from Fisher 344 rats livers, both with and without phenobarbital pretreatment, to 1.5% halothane or 2.0% isoflurane in 1%, 2%, or 4% (control) oxygen. Isoflurane exposure was included for a control of anesthetic effects on hepatocytes, because it is known to be metabolized minimally and probably is not associated with hepatic dysfunction. Oxygen levels were chosen to approximate those that may occur in the liver in vivo. Cell death was assayed via aspartate aminotransferase (AST) release, both immediately following a 2-h oxygen ± anesthetic exposure and 6 h post-exposure. Per cent cell death data were analyzed using multiple regression techniques. Results obtained immediately, and 6 h after exposure demonstrate that low oxygen levels, halothane, and phenobarbital were each highly significant factors (P < .001) in relation to cell death, in agreement with the halothane-phenobarbital-hypoxia rat model. A toxic effect of isoflurane was not observed under identical experimental conditions. The results of the study clearly indicate that the origin of cell death in hepatocyte monolayers is multifactorial; hypoxia, phenobarbital induction, and halothane exposure each contribute to the hepatocyte damage observed in our in vitro model. (Key words: Anesthetics, volatile: halothane; isoflurane. Biotransformation: enzyme induction; metabolites; microsomes; phenobarbital. Hypoxia; liver; reperfusion. Liver; hepatotoxicity; hypoxia; metabolism. Toxicity: hepatic.)

The rat model of halothane hepatotoxicity developed by Widger et al.,1 McChin et al.,2 and Ross et al.3 requires that halothane anesthesia be administered in hypoxic gas mixtures to rats whose liver microsomal enzymes have been induced by phenobarbital. Halothane administered in this context leads to necrosis in zone three of the hepatic acinus within 24 h post-exposure,4 particularly in the Fisher 344 rat strain. A number of theories have been advanced to explain the etiology of halothane hepatotoxicity that occurs in the phenobarbital-hypoxia rat model. Perhaps the most extensively studied hypothesis maintains that halothane is metabolized to chemically reactive species by liver microsomal P-450, which is greatly increased by phenobarbital pretreatment, and that these metabolites may subsequently destroy liver parenchyma.5,6,6 Halothane is metabolized via reductive pathways under conditions of hypoxia, thereby generating products such as chlorodifluoroethylene, which may bind to microsomal proteins and glutathione.5,10 Hepatic injury may be reduced by inhibitors of the cytochrome P-450 complex.11,11 Halothane biotransformation may also generate toxic free radical species, such as the CF3CHCl radical,5,6,8 which interact with unsaturated fatty acids of membrane phospholipids and may lead to lipid peroxidation, provided a moderately hypoxic environment is present (fig. 1).9,10 Halothane, phenobarbital induction, and hypoxia are essential to the metabolic hypothesis, and are all present in the rat model.

Anesthetic metabolism alone, however, is not necessarily the cause of hepatic injury which may occur postoperatively. For example, Van Dyke13,14 found that centrilobular necrosis may occur in rats anesthetized with enfurane and isoflurane. Subsequently, it has been demonstrated that these anesthetics are metabolized minimally when compared to halothane,15 and not via reductive pathways. Anesthetics may also reduce hepatic blood flow,15 leading to hypoxia and intracellular energy deficits (fig. 1).17 Shingu et al.18 reported that hypoxia alone may account for the hepatotoxicity seen in the phenobarbital-hypoxia rat model; they suggested that hypoxia may be more important than metabolism of halothane in the pathogenesis of liver injury.17 Separation of the hypoxic and metabolic effects of halothane is impossible in an in vivo experimental system, since reductive metabolism requires hypoxia. In

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the present experiments, confluent monolayers of rat hepatocytes were used to elucidate the contribution to hepatotoxicity of each factor in the phenobarbital-hypoxia model. Because it is metabolized less than 1% as much as halothane,15 isoflurane was used as a control for possible adverse effects of anesthetics other than metabolism, such as uncoupling of mitochondria, alteration of electron transport chains, and increased transmembrane ion leakage. Cultured hepatocytes are ideal for this investigation, since oxygen concentrations can be uniformly controlled, eliminating the oxygen gradient known to exist between periportal and pericentral regions in the hepatic acinus,19 as well as the effects of halothane and isoflurane on cardiac function and hepatic blood flow.16-18 An advantage of performing these experiments on monolayer cultures versus suspensions of primary hepatocytes is that a single layer of cells attaches directly to highly gas-permeable plastic (Lux Pernanox®, Flow Laboratories). The manufacturer has shown that these dishes reach equilibrium with the gases and anesthetics very rapidly without concentration gradients between the cells.

Hepatocytes of phenobarbital pretreated and non-pretreated Fisher 344 rats were exposed to halothane or isoflurane for 2 h in hypoxic gas mixtures. Cell death was assayed immediately and 6 h post-exposure. Oxygen concentrations were chosen at 1%, 2%, and 4%, in order to approximate the physiologic spectrum of hypoxia to normoxia in the liver lobule.19,20 and to encompass experimental precedents established by other in vitro halothane metabolism studies.21-22 Previous studies in our laboratory showed that 1% oxygen for 2 h was the lowest concentration that was only moderately toxic to the controls, whereas 4% oxygen is more appropriate for simulating in vivo conditions.23,24

Materials and Methods

Hepatocyte Isolation

Adolescent male Fisher 344 rats (180–240 g) were utilized for all experiments. Animals pretreated with phenobarbital received intraperitoneal injections (80
mg/kg/day) on each of four consecutive days prior to hepatocyte isolation. Hepatocytes were prepared by in situ perfusion of the liver with collagenase. Each rat was anesthetized with pentobarbital (50 mg/kg), the peritoneum was opened, an 18-gauge catheter was inserted into the portal vein, the inferior vena cava was severed, and the liver was perfused with EGTA-containing buffer for 4 min at a flow rate of 25 ml/min to remove blood, calcium, and collagenase inhibitors. Collagenase Type IV (Sigma) solution was then perfused for 10 min at the same flow rate. Following collagenase perfusion, the liver was excised and gently raked to release hepatocytes. The hepatoma were swirled in a DNase-containing buffer, filtered, and washed three times by centrifugation at 10 × g.

The isolated and washed hepatocytes were suspended in 100 ml of a medium (complete M-199) containing Gibco M-199 with Earle’s salts, pH 7.3, 100 U penicillin/ml, 100 μg streptomycin/ml, 4.5 μg gentamicin/ml, 1 μg transferrin/ml, 0.25 mM ascorbic acid, 4.12 μM folic acid, 1.24 mM pyruvate, 8 μg insulin/ml, 100 μM 5-aminolevulinic acid, 0.25% bovine serum albumin, and 10% heat inactivated fetal calf serum (Gibco). The 5-aminolevulinic acid has been shown to maintain total cytochrome P-450 content at in vivo levels. One-milliliter aliquots containing approximately 3 × 10⁶ cells were added to collagen-coated 60-mm Lux Permanox Contur (Flow) culture dishes, and allowed to attach for 90 min at 37°C in 5% CO₂/95% air. These culture dishes were pre-incubated for 3 h in complete M-199 with 20% fetal bovine serum and 1 μM dexamethasone to form a matrix, which facilitates attachment, prior to addition of cell suspension aliquots. The high oxygen permeability of the Permanox dishes permits rapid equilibration of the cells and medium with any desired gaseous environment. The hepatocyte monolayers were then washed twice after the 90-min attachment period, and covered with 2 ml complete M-199. Cells at this point were more than 95% viable as assayed 5–15 min after addition of Trypan Blue by observing five fields of approximately 200 cells at 200× magnification with a Nikon Diaphot inverted microscope.

In a separate test of cytochrome P-450 content, hepatic microsomes were prepared by scraping hepatocytes from 14 plates with a rubber policeman 24 h after isolation, homogenizing with a micro Potter-Elvich homogenizer, purifying by differential ultracentrifugation, and finaly suspending in 0.02 M Tris-HCl buffer at pH 7.4. Microsomal protein was determined by the BioRad assay using bovine serum albumin as a standard. Each dish contained an average of 1.63 × 10⁶ cells and 46 μg microsomal protein. The concentration of microsomal cytochrome P-450 was determined from the difference spectrum of carbon monoxide bound to reduced cytochrome P-450 versus reduced cytochrome P-450. The average level of total cytochrome P-450 in the untreated cells was 0.8 nmoles P-450/mg microsomal protein; in animals pretreated with phenobarbital, hepatocyte P-450 levels rose to 2.5 nmoles P-450/mg microsomal protein. These levels are in agreement with non-induced and phenobarbital-induced values. Previous studies in this laboratory, involving identically prepared hepatocytes, demonstrated the metabolic activity of these cells before, during, and after 3.5 h of 2% O₂ hypoxia.

**Experimental Protocol.**

Halothane and isoflurane exposure experiments were initiated 18 h following isolation and plating of the hepatocytes. Figure 2 shows a flow chart of the experimental protocol. The hepatocyte monolayers were separated first into two groups according to phenobarbital or no pretreatment. Each of these groups was then randomly divided into six groups of six dishes each for exposure to 2 h of 1%, 2%, or 4% oxygen: three with either 1.5% halothane or 2.0% isoflurane, and three without anesthetic. The MAC value for halothane is 0.95–1.1% in the rat, somewhat higher than for humans. Since clinical anesthesia is frequently administered at approximately 1.5 times MAC, concentrations of 1.5% halothane and 2.0% isoflurane were used in this study. Prior to each anesthetic or control exposure, the monolayers were washed twice with buffer and covered with 2 ml complete M-199 without ascorbic acid, dexamethasone, albumin, or fetal bovine serum, and checked for confluency with an inverted microscope.

Cells were then removed from a standard incubator (5% CO₂/95% air at 37°C) and placed in a Billups-Rothenberg (Del Mar, CA) modular incubator chamber for exposure to halothane or isoflurane and selected hypoxic gas mixtures. The incubator chamber was altered to permit the gas mixture to flow in from the top of the chamber onto the surface of a 100-mm petri dish filled with sterile H₂O that was placed on the top shelf. The resulting turbulence caused a uniform gas flow over six culture dishes placed on each of the two lower shelves, with phenobarbital pretreated and untreated dishes randomly intermixed. The outflow port at the incubator chamber was extended upward to the surface with tubing, and the entire sealed incubator chamber was immersed in a 37°C water bath.

The gas mixtures of either 1%, 2%, or 4% O₂/5% CO₂(balance N₂ were made in a standard anesthesia gas machine (Foregger Instruments, Roslyn Heights,
Fig. 2. Experimental protocol. One rat of a pair of adolescent male Fisher 344 rats was pretreated for 4 days with interperitoneal phenobarbital injections. On the fifth day, hepatocytes were prepared from both rats by in situ collagenase digestion. The hepatocytes were incubated into culture dishes and formed confluent monolayers of hepatocytes within 18 h. At this point, they were divided into 12 treatment groups according to oxygen concentration, anesthetic exposure, and phenobarbital pretreatment. After the 2-h exposure period, they were further divided into groups that were evaluated for toxicity immediately or after 6 h in an incubator. Each of the resulting 24 experimental groups contained three to five culture dishes of hepatocyte monolayers. The experiment was repeated seven times.

NY) and were flowed at 4 l/min through a Fluotec vaporizer chamber (Fraser, Buffalo, NY) calibrated to form mixtures of 1.5% halothane or 2.0% isoflurane with a Puritan-Bennett anesthetic vapor analyzer, model 222. A metering valve was used to direct 1 l/min of this flow through a glass frist humidifier that humidified and warmed the gas mixture to 37°C before entry into the exposure chamber. An oxygen monitor (OHMEDA 5100, B.O.C. Health Care, Madison, WI) calibrated against room air and various O2/N2 mixtures was placed in the incubator and used to maintain the desired O2 concentrations. A separate trial experiment (data not shown) demonstrated that, with gentle rocking of the chamber during the initial 10 min of the experiment, 1%, 2%, and 4% O2 concentrations in the 2 ml of culture media in the Permanox dishes were obtained at 20, 8, and 5 min, respectively. All groups of dishes remained in the incubator chamber for a 2 h exposure with lids off, and experiments in the six groups were performed sequentially throughout the day.

Immediately following the experimental exposure, supernatants from three of the six monolayers in each experimental group were removed, centrifuged to separate dead cells and debris, and the resulting supernatant analyzed for aspartate aminotransferase (AST) release using Sigma Kit No. 505 (Sigma Chemical Co., St. Louis, MO). This assay is similar to measurement of SGOT in vivo. A supplementary assessment of cell death was obtained by measuring Trypan Blue exclusion of the cells following removal of the supernatant. The remaining three plates in each group were incubated in 5% CO2/95% air at 37°C for 6 h. At the end of this 6-h period (Time 6), supernatants were tested for AST release and the hepatocyte monolayers for Trypan Blue exclusion, as described above. A separate set of non-anesthetic exposed plates were used as controls during the isoflurane experiments, and the isoflurane results were analyzed within this group separately. There was no statistical difference in AST release between the non-exposed hepatocytes in the two groups.

DATA ANALYSIS

Experiments were performed on seven separate days to average dish-to-dish and rat-to-rat variations with an untreated and a phenobarbital-pretreated rat each day. Occasional technical problems caused some data points
Fig. 3. Per cent cell death, measured immediately after a 2-h exposure period (Time 0), as a function of oxygen concentration (% O₂), control exposure (C), exposure to halothane (H), pretreatment with phenobarbital (P), or combined exposure to halothane and pretreatment of the rat with phenobarbital (HP). The number (n) of hepatocyte monolayers examined for each condition is shown below each column.

to be lost or rejected, yielding slightly varying group sizes (figs. 3–6). Data were collected as per cent of cells dead; AST release for each dish was compared to complete AST release caused by lysis with 0.2% Triton X-100, and expressed as a per cent. Estimates of Trypan Blue uptake were used for comparison with AST measurements of cell death, but were not entered into the data analysis directly because of their greater variability and subjectivity. In previous studies, we have shown an excellent correlation between ⁵¹Cr leakage and AST release as a measure of cell death.²⁰,²³ Multiple regression²⁰,²¹ was chosen as the method of data analysis because of the study design which included multiple independent (X) variables and a single dependent (Y) variable. Multiple regression was also appropriate due to the varying numbers of observations in experimental treatment conditions. Independent (X) variables included halothane, oxygen concentration, phenobarbital induction, and their first and second order interaction terms.

Per cent cell death values (P) represented the dependent variable, and were converted to logit values (Y) where Y = ln (P/100-P) for data analysis.²¹ Oxygen levels were coded ½, 0, and −½ to represent 1%, 2%, and 4% oxygen in the gas mixture; yes or no in regard

Fig. 4. Per cent cell death, measured 6 h after the end of a 2-h exposure period (Time 6), as a function of oxygen concentration (% O₂), control exposure (C), exposure to halothane (H), pretreatment with phenobarbital (P), or combined exposure to halothane and pretreatment of the rat with phenobarbital (HP). The number (n) of hepatocyte monolayers examined for each condition is shown below each column.

Fig. 5. Per cent cell death, measured immediately after a 2-h exposure period (Time 0), as a function of oxygen concentration (% O₂), control exposure (C), exposure to isoflurane (I), pretreatment with phenobarbital (P), or combined exposure to halothane and pretreatment of the rat with phenobarbital (IP). The number (n) of hepatocyte monolayers examined for each condition is shown below each column.
to phenobarbital induction or anesthetic exposure per trial were coded $\frac{1}{2}$ and $-\frac{1}{2}$, respectively. Using these variables, an orthogonal design was created for the regression equation. Treatment group sizes, although unequal, were sufficiently equal to approximate an orthogonal solution. The data were analyzed using StatView 512+ software on an Apple Macintosh 512K computer to obtain estimates of the coefficients A, B, C, and D in the following equation, and to obtain tests of whether each was significantly different from zero (no effect):

$$Y = A + B_1X_1 + B_2X_2 + B_3X_3 + C_{41}X_1^2 + C_{12}X_1X_2 + C_{13}X_1X_3 + C_{23}X_2X_3 + DX_1X_2X_3,$$

where $Y = \ln (P/100-P)$, $X_1 = \text{oxygen (} \frac{1}{2}, 0, -\frac{1}{2})$, $X_2 = \text{phenobarbital (} -\frac{1}{2}, \frac{1}{2})$, and $X_3 = \text{halothane (} -\frac{1}{2}, \frac{1}{2})$.

**Results**

Results of the study are shown in figures 3–6, as well as in tables 1–4. Mean differences between experimental treatment conditions using halothane at Time 0 are presented in figure 3. The mean percent of dead cells per monolayer increases upon moving from 4% to 1% oxygen, from no phenobarbital pretreatment to phenobarbital pretreatment, and from no halothane-to-oxycarb-mhalothane exposure. Cell death reaches nearly 75% in all of the 1% oxygen treatment groups. This value seems to represent a plateau of AST release due to experimentally induced lysis compared to 0.2% Triton X-100 lysis. As a result, no additivity was observed. In separate experiments (data not shown), hepatic cell monolayers maintained for 4 h at 20%, 12%, or 4% oxygen were comparably viable as measured by Trypan Blue assay and the release of AST, demonstrating that 4% oxygen is an adequate control level for oxygen in this study.

The trends in experimental means in figure 3 were tested for significance in the multiple regression analysis (table 1). The overall correlation squared ($R^2$) given at the top of the table represents the amount of variance that is accounted for by the regression equation. $R^2$ in this case is approximately 80%, showing that the three independent variables and their interactions explain a substantial amount of the observed variance, which is an
amount adequate for interpretation. The probability of obtaining a correlation of .89 due to chance factors alone is less than 1.001. Table 1 contains the coefficient values of the variables and their interactions. Labels in parentheses for coefficient values are taken from the complete regression equation (see Materials and Methods). The probabilities of the beta coefficients reveal that the individual effects for oxygen, phenobarbital, and halothane are highly significant ($P < 0.001$). This probability reflects the likelihood that the $\beta$-coefficient value is equal to zero (no effect). Residuals from the multiple regression were also examined, and the fit was satisfactory.

The logit value of the interaction of phenobarbital induction and halothane exposure (table 1) is $-1.52$, signifying that less cell death occurs when phenobarbital and halothane are present together than would be predicted from their individual contributions to cell death. One cannot infer that the combined effect is protective; rather, the interaction reveals that the effects are not additive, because the rate of cell death is nearly maximal for both halothane and phenobarbital. Similarly, the three-way interaction, which represents complete summation of toxic effects in the metabolic hypothesis, is clearly not significant. Again, the failure to observe complete additivity of effects is most likely due to the great toxicity of each of the individual factors at 1% oxygen.

Six hours following halothane/hypoxia exposure (Time 6), trends identical to those seen at Time 0 are reflected in the means for oxygen, phenobarbital, and halothane (fig. 4). Again, these effects and the overall solution to the regression are highly significant, with 79% of the variance explained by the solution (table 2). Time 6 data show a number of significant interactions which elucidate the role of oxygen in this study. The oxygen/oxygen self-interaction term (table 2, line 4) was entered into the regression analysis to describe more accurately the effect of oxygen. The oxygen/oxygen effect, which is highly significant ($P < 0.001$), suggests a quadratic, or nonlinear, relation. This quadratic term may be interpreted as an overwhelming effect of 1% oxygen on cell death, which drops markedly to 2% and then levels off at 4% in a parabolic fashion. Because this oxygen effect is so powerful, it approaches significance in the oxygen/halothane interaction ($P = 0.086$), and reaches significance between oxygen and phenobarbital ($P < 0.05$).

Results of the isoflurane exposure experiments are presented in figures 5 and 6, as well as in tables 3 and 4. Trends identical to those observed in the halothane exposure groups are repeated for the oxygen and phenobarbital variables, with hypoxia and pretreatment with phenobarbital contributing significantly to cell death.

### Table 3. Multiple Regression Analysis with Eight Variables at Time 0 (See Text)

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Beta Coefficient Value</th>
<th>Standard Error</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (A)</td>
<td>-2.515</td>
<td>.334</td>
<td>.0001</td>
</tr>
<tr>
<td>Oxygen (b$_1$)</td>
<td>2.842</td>
<td>.273</td>
<td>.0001</td>
</tr>
<tr>
<td>Phenobarbital (b$_2$)</td>
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<td>.0001</td>
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<tr>
<td>Isoflurane (b$_3$)</td>
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<td>.273</td>
<td>.3273</td>
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<tr>
<td>Oxygen/oxygen (C$_{11}$)</td>
<td>3.424</td>
<td>1.16</td>
<td>.0039</td>
</tr>
<tr>
<td>Oxygen/phenobarbital (C$_{12}$)</td>
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<td>.67</td>
<td>.0017</td>
</tr>
<tr>
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<td>.1433</td>
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<tr>
<td>Oxygen/isoflurane (C$_{33}$)</td>
<td>-.295</td>
<td>.67</td>
<td>.6602</td>
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<tr>
<td>Oxygen/phenobarbital/isoflurane (D)</td>
<td>.332</td>
<td>1.339</td>
<td>.8049</td>
</tr>
</tbody>
</table>

Degrees of freedom = 107; $R = .73$; $R^2 = .533$; $P < 0.001$.
* Probability that the coefficient value is equal to zero (no effect).

Without the toxic effects of halothane, the interaction terms of oxygen/oxygen (tables 3, 4, line 4) (see previous paragraph) and of phenobarbital/oxygen (tables 3, 4, line 5) are significant at Times 0 and 6, an effect which only became apparent at Time 6 in the presence of halothane. Tables 3 and 4 quantify these effects of oxygen and phenobarbital, which are similar in magnitude and direction to those in the halothane experiments. However, coefficient values (tables 3, 4) for isoflurane and its interactions with oxygen (line 7) and phenobarbital (line 6) indicate that isoflurane (line 3) is clearly not a hepatotoxin in this setting; cell death can be attributed exclusively to oxygen, phenobarbital, and their interactions when isoflurane is used.

### Discussion

The results of this study indicate that three essential components of the rat model for halothane hepatotox-
icity, namely phenobarbital induction (PB-I), hypoxia, and halothane exposure, each contribute significantly to hepatocyte necrosis in our in vitro system. The study shows clearly that halothane hepatotoxicity cannot be ascribed to anesthetic-induced hypoxia alone. In addition, isoflurane exposure does not affect cell viability, thus eliminating general anesthetic effects as contributors to hepatotoxicity in this study.

The overwhelming effect of hypoxia at both Time 0 and Time 6 agrees with the findings of earlier studies,14,17,18 in that 1% oxygen in the absence of anesthetic exposure results in substantial cytotoxicity (tables 1–4). Because neither respiratory depression,25 decreases in hepatic blood flow,16,34 nor a depleted nutritive status15,38 are involved in the generation or exacerbation of hypoxia in this study, the source of cellular hypoxia is direct and consistent at the lowest oxygen level. Hypoxia is known to induce energy deficits,17,19,22 such as a reduction in ATP/ADP or pyruvate/lactate ratios, which might be responsible for the cytotoxic effect. In addition, we have previously shown that hypoxia compromises the ability of the hepatocyte to maintain correct calcium compartmentalization25 and decreases the LD₅₀ of tert-butylhydroperoxide in hepatocytes by more than an order of magnitude.25

The interactive effects of oxygen at Time 6 are more pronounced than at Time 0 (tables 2, 4). We have previously observed the toxic effects of reoxygenation on hypoxic hepatocytes.25 It is likely that this damage is similar to the injuries observed upon re-oxygenation of brain, intestine, and cardiac tissue.25 The damage due to generation of free radical species and formation of lipid peroxides10,36 during the hypoxic period5,8,21 may be exacerbated upon reoxygenation by a sudden production of superoxide radical anion. During hypoxia of tissue, the dehydrogenase form of xanthine oxidase is transformed to the oxidase form concomitant with an accumulation of hypoxanthine. Upon reperfusion of the tissue, the availability of O₂ and hypoxanthine as co-substrates results in xanthine oxidase-dependent formation of large amounts of superoxide radical anions which undergo iron-catalyzed (Fenton-type) reactions to yield highly reactive hydroxyl radicals.35

The significant effect of phenobarbital pretreatment in relation to cell death is somewhat surprising; the result implies that phenobarbital itself is hepatotoxic. Yates et al.37 hypothesized that phenobarbital induction may be important in the rat model because it increases tissue mass, and may, thereby, increase cellular oxygen demand. For cells in monolayer culture, however, approximately equal numbers of cells are present in culture dishes from phenobarbital-induced and non-induced animals. It should be emphasized that the pheno-
volved in this study, because of the immediacy of cell death and lack of complement components in the culture medium.

In conclusion, although hypoxia alone does result in severe hepatotoxicity as previously suggested,16–18 there is clearly an additional toxicity due wholly to halothane exposure and phenobarbital pretreatment. The experimental system was designed to eliminate confounding variables, such as concomitant reductions in hepatic blood flow, changes in blood oxygen saturation, alterations of the proportion of portal vein to hepatic artery perfusion, or changes in the slope of the zone 1 to zone 3 oxygen gradient. The absence of increased toxicity in the groups exposed to isoflurane implicates halothane metabolism as a factor in the pathogenesis of injury.

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