

## Halothane Decreases Albumin and Transferrin Synthesis: Studies in the Isolated, Perfused Rat Liver and in the Intact Rat

J. J. Franks, M.D.,\* J. B. Kruskal, M.B., Ch.B., Ph.D.,† R. E. Kirsch, M.D.,‡ A. P. G. Beechey, M.B., Ch.B.,§  
D. F. Morrell, M.B., Ch.B.,¶ G. G. Harrison, M.D.\*\*

Isolated perfused rat livers exposed to 1.5% halothane (equivalent to 1.35 MAC) in O<sub>2</sub>/CO<sub>2</sub> or to O<sub>2</sub>/CO<sub>2</sub> alone produced urea, as well as albumin and transferrin (both measured by immunodiffusion), at constant rates during a 4.25-h perfusion. Urea production did not differ in the two treatment groups, but halothane depressed albumin and transferrin synthesis 43% and 45%, respectively. Intact rats were also exposed to halothane, after which albumin synthesis was measured by the (<sup>14</sup>C)carbonate technique. The minimum halothane concentration required to insure sufficient relaxation for ventilation was selected and ranged from 1.0 to 1.5%. Measurements were made in control rats not exposed to halothane (group I) and in halothane exposed rats immediately after 1 h of anesthesia (group II), 24 h after the start of 1 h of anesthesia (group III), and immediately after ½ h of anesthesia preceded by a 1-h exposure 24 h earlier (group IV). Single exposures to halothane (groups II and III) resulted in a decrease in albumin synthesis immediately or 24 h later that did not differ significantly from controls (group I). However, halothane given twice to rats at 24-h intervals (group IV) reduced their mean albumin synthesis rate to half that of controls. The early onset and constancy of halothane depression of export protein synthesis by isolated, perfused livers may reflect a response to halothane itself, rather than an effect resulting from the accumulation of halothane metabolites. Similarly, reduction of albumin synthesis in intact rats immediately after a second halothane exposure may indicate a response to halothane, rather than to halothane metabolites. (Key words: Anesthetics, volatile; halothane. Liver: albumin synthesis; transferrin synthesis; urea production. Toxicity: halothane.)

HEPATIC DYSFUNCTION of varying degree has been attributed to exposure to most volatile anesthetics, especially halothane,<sup>1-4</sup> but the only abnormality reported to occur regularly after halothane anesthesia is delayed

BSP excretion.<sup>5</sup> Histologic and functional derangements can be produced by hormonal and/or pharmacologic manipulation of intact experimental animals anesthetized with halothane,<sup>6-8</sup> and changes in carbohydrate and protein metabolism in perfused rat livers<sup>9-11</sup> and in isolated rat hepatocytes<sup>12</sup> have been observed when these preparations were exposed to halothane.

One major function of the liver is synthesis of proteins for use in the liver itself or elsewhere in the organism. We have, therefore, examined the effect of a clinically relevant level of halothane (equivalent to 1.35 MAC) on albumin and transferrin synthesis by the isolated, perfused rat liver. We also examined the effect of halothane on the synthesis of albumin in the intact rat.

### Materials and Methods

#### ANIMALS

Animal use was approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town. Male Black-hooded rats of the Long Evans strain weighing 296-305 g, housed under controlled conditions of temperature and humidity and allowed free access to food and water at all times, were used as liver and red cell donors. Male rabbits of the New Zealand White strain, maintained under the same conditions, were used as plasma donors. Rats used for (<sup>14</sup>C)carbonate studies were, in addition, given drinking water containing 0.008M NaI for at least 24 h before injection. Animals in this group weighed between 275 and 325 g.

#### LIVER PERFUSIONS

Considerations and techniques for perfusing isolated rat livers have been reviewed recently.<sup>13</sup> For our experiments, livers were perfused<sup>14-16</sup> with a heterologous mixture<sup>17</sup> consisting of heparinized rabbit plasma and rat erythrocytes washed twice with 0.9% saline. Perfusate volumes ranged from 113 to 144 ml, with hematocrits of 25-29%. The perfusate was exposed to O<sub>2</sub>/CO<sub>2</sub> (95/5) by circulation in a warmed (37° C), humidified perfusion cabinet for 0.5 h before insertion of the liver and throughout a 4.25-h period of liver perfusion. Before placement in the perfusion chamber, the liver was flushed with isotonic crystalloid (Plasmalyte A, Tra-

\* Professor of Anesthesiology, Vanderbilt University.

† Research Fellow in Medicine, Department of Medicine, University of Cape Town.

‡ Professor of Medicine, Department of Medicine, University of Cape Town.

§ Lecturer in Anaesthetics, Department of Anaesthetics, University of Cape Town.

¶ Senior Lecturer in Anaesthetics, Department of Anaesthetics, University of Cape Town.

\*\* Professor and Chairman, Department of Anaesthetics, University of Cape Town.

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Address reprint requests to Dr. Franks: Department of Anesthesiology, Vanderbilt University, Nashville, Tennessee 37232.

TABLE 1. Schematic of Protocol

Group	Time of Halothane Exposure		Time of Measurement of Albumin Synthesis
	First (1.0 h)	Second (0.5 h)	
I	—	—	0 h
II	0 h	—	1 h
III	0 h	—	24 h
IV	0 h	24 h	24.5 h

venol, Deerfield, IL) for 2 min at a pressure of no more than 15 cm of H<sub>2</sub>O. The perfusion mixture was then pumped through the portal vein in an antegrade direction, with pressure maintained at 12–13 cm of H<sub>2</sub>O. In control perfusions, plasma donor rabbits and red cell and liver donor rats were anesthetized briefly with ether in O<sub>2</sub>/CO<sub>2</sub>, whereas, in halothane experiments, 1.5%, *i.e.*, 1.35 MAC<sup>18</sup> halothane, was used. In addition, in halothane experiments, the perfusate was exposed to 1.5% halothane in O<sub>2</sub>/CO<sub>2</sub> throughout the 0.5-h preparatory and 4.25-h study periods. Perfusate flow and bile production were monitored continuously. Perfusate blood gases were sampled distal to the liver in the perfusate circuit, *i.e.*, on the “venous” side, at 0.5-h intervals. pO<sub>2</sub> was maintained at >90 mmHg, pCO<sub>2</sub> between 34 and 38 mmHg, and pH between 7.35 and 7.44, the last by addition, when needed, of 1.0–3.0 ml of 0.5M NaHCO<sub>3</sub>. Perfusate hematocrit was measured before and at the end of liver perfusion, whereas samples for the measurement of albumin, transferrin, and urea<sup>19</sup> were taken before insertion of the liver and 0.25, 0.75, 1.25, 1.75, 2.25, 3.25, and 4.25 h after the start of hepatic perfusion. Estimates of total albumin, transferrin, and urea production were adjusted for loss due to sampling. The concentrations of albumin and transferrin produced by the liver were measured by radial immunodiffusion.<sup>20</sup> The antisera used (Cooper Diagnostics, Malvern, PA) reacted with rat but not rabbit albumin or transferrin. Urea production and albumin and transferrin synthesis rates were estimated by linear regression<sup>21</sup> of accumulated values, with rates taken as equal to the slopes of the fitted lines. Rates for the 4.25-h perfusion period were expressed as mg · h<sup>-1</sup> per 300 g rat. Results in the control and halothane treated groups were compared by Student's *t* test.

#### INTACT RATS

Many of the methods have been described before.<sup>22–24</sup> Usually, two rats were brought from the animal quarters each experimental day at 0700. Table 1 shows a schematic of the experimental protocol, indicating each treatment group, the hour that the rats in each group were anesthetized, and the hour that albumin synthesis was measured in rats in each treatment group. The control animal (group I) was lightly anesthe-

tized with diethyl ether in O<sub>2</sub>/CO<sub>2</sub> (95/5) and injected intravenously at the base of the tail with a mixture of 200 μCi of sodium (<sup>14</sup>C) carbonate<sup>25</sup> and 10 μCi of <sup>125</sup>I-albumin. Ether anesthesia was stopped after this injection, with total ether exposure time lasting less than 5 min. The rat was returned to a cage with free access to food and water. Blood samples were collected from the tip of the tail at 0.25, 4, 4.5, 5, and 5.5 h after injection. At 6 h, the rat was anesthetized with ether and bled by cardiac puncture. The experimental rat (group II, III, or IV) was anesthetized with halothane in warmed, humidified air/oxygen (FI<sub>O<sub>2</sub></sub> = 0.4). Body temperature was maintained with the aid of a heat lamp. EKG monitoring revealed no detectable changes or abnormalities during the anesthetic period. The oxygen/air mixture was obtained from compressed gas sources and delivered *via* a rotameter bank to a halothane plenum vaporizer. The anesthetic mixture was delivered at 4 l · min<sup>-1</sup> *via* a T-piece circuit to a Tygon cylinder (d = 2.5 cm, h = 5 cm) stoppered at one end. The gases entered through a lateral opening in the cylinder and exited through an opening at the stoppered (distal) end. With the rat in a prone (physiologic) position, the cylinder fit snugly over the muzzle and, when held in place with both hands, thumbs thrusting the mandible down and forward, delivered, under careful observation, an adequate tidal volume at 55–60 b/min. Intermittent positive pressure in the system was developed by occluding gas outflow with a solenoid valve. Occlusion was carried out manually by an assistant or with a foot switch. Between 1.0 and 1.5% halothane was used; the minimum concentration necessary to insure sufficient relaxation for ventilation was selected. (Ventilation was assessed in a pilot experiment in three animals anesthetized with halothane, but otherwise excluded from the study. Thirty and 60 min after the start of anesthesia, femoral arterial blood samples revealed pH, PaO<sub>2</sub>, and PaCO<sub>2</sub> in the normal range.) After 1 h of halothane anesthesia, the experimental rat was injected and samples taken (group II) as described for the control rat, with cessation of anesthesia after isotope injection. Alternatively, the experimental rat was anesthetized with halothane for 1 h without injection and sampling, and returned to a cage with access to food and water. Twenty-three hours later, the animal was either anesthetized briefly with ether (group III) and otherwise treated like groups I and II, or anesthetized for 0.5 h with halothane (group IV) and otherwise treated like groups I and II. In all rats, <sup>125</sup>I-albumin activities were measured in the 0.25- and 6-h plasma samples for determination of plasma volume and to provide a correction factor for loss of labeled plasma albumin by catabolism and transfer to interstitial fluids. Urea carbon specific activity was measured in the 4–5.5-h samples. The values for each animal were plotted against time, fitted by a single

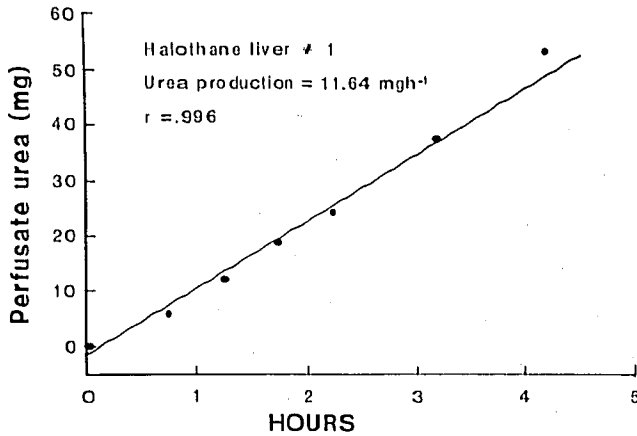


FIG. 1. Urea production by an isolated perfused rat liver exposed to 1.5% (equivalent to 1.35 MAC) halothane. Each data point represents the total amount of perfusate urea less the pre-perfusion value. Urea production rate is derived from the slope of the regression line and expressed per 300 g rat.

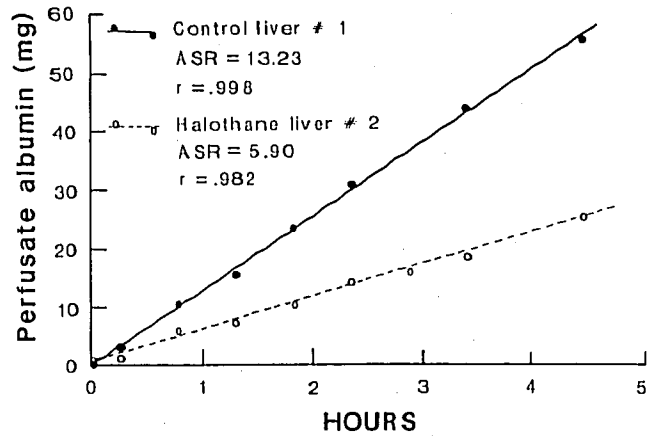


FIG. 2. Albumin synthesis rates per 300 g rat (ASR) by isolated perfused rat livers with and without exposure to 1.5% halothane. Data points and synthesis rates were derived as described in figure 1.

exponential function, and corrected by extrapolation to zero time ( $t_0$ ). Albumin and urea concentrations and arginine-6-carbon specific activity in albumin were measured in the 6-h sample,<sup>21</sup> and the latter was corrected to  $t_0$  by multiplying by the albumin loss-correction factor. From these data, the albumin synthesis rate at  $t_0$ , in  $\text{mg} \cdot \text{h}^{-1}$  per 300 g rat, was calculated for each animal.<sup>23</sup>

Control and treatment groups were compared by one-way analysis of variance, and by the Student-Newman-Keuls multiple comparison procedure.<sup>21</sup> Statistical significance was inferred if  $P < 0.05$ .

## Results

### PERFUSION STUDIES

Perfusate flow, in  $\text{ml} \cdot \text{m}^{-1}$  per g liver, ranged from 1.7 to 3.9 in control livers and from 1.6 to 3.5 in halothane-treated livers. Bile flow, in  $\mu\text{l} \cdot \text{m}^{-1}$  per g liver, ranged from 1.5 to 1.9 in control livers and from 1.1 to 2.2 in halothane-treated livers. Halothane perfusate and bile flow rates did not differ significantly from corresponding control values.

Urea production (mean  $\pm$  SEM) was  $11.36 \pm 1.17$  and  $13.26 \pm 1.01 \text{ mg} \cdot \text{h}^{-1}$  per 300 g rat in control and halothane livers, respectively. These means do not differ significantly. Figure 1 shows a plot of urea production from a typical halothane liver perfusion. A constant rate of urea production over 4.25 h was a characteristic finding in all experiments, with and without halothane exposure.

Figure 2 shows plots of albumin synthesis by representative control and halothane-treated livers. Again, a constant rate of production was found throughout the

course of each liver perfusion. Similarly, transferrin was synthesized at a constant rate during each perfusion. However, the overall rate of synthesis of each of these export proteins was significantly depressed by halothane exposure. Table 2 shows the mean and standard error for albumin and transferrin synthesis by isolated, perfused rat livers exposed to  $\text{O}_2/\text{CO}_2$  (control) and to  $\text{O}_2/\text{CO}_2$  plus 1.5% halothane for 4.25 h. Halothane reduced albumin synthesis by 43% and transferrin synthesis by 45%. Albumin synthesis in control livers approximated that observed in intact rats as measured by the ( $^{14}\text{C}$ )carbonate method and by extrapolation from catabolic data.<sup>22-24</sup> The transferrin synthesis rate, measured in rats by isotopic techniques, has been reported to be about one-fifth that of albumin,<sup>26-27</sup> but rates measured in our control liver preparations averaged closer to one-third the albumin synthesis rate.

### INTACT RAT STUDIES

Table 3 shows the mean and standard error for the albumin synthesis rate in each treatment group. A single halothane exposure did not significantly decrease albumin production either immediately or 24 h later (groups II and III). However, halothane given twice to rats in a 24-h interval (group IV) reduced their mean

TABLE 2. Effect of Halothane on Albumin and Transferrin Synthesis by the Isolated, Perfused Rat Liver

Group (n)	Protein Synthesis ( $\text{mg} \cdot \text{hr}^{-1}$ per 300 g rat)	
	Albumin	Transferrin
Control (6)	$12.42 \pm 1.15^*$	$4.35 \pm 0.75$
Halothane (5)	$7.10 \pm 0.73^\ddagger$	$2.40 \pm 0.17^\ddagger$

\* Mean  $\pm$  SEM.

† Differs from control ( $P < 0.01$ ).

‡ Differs from control ( $P < 0.02$ ).

TABLE 3. Effect of Halothane on Albumin Synthesis in the Intact Rat

Group (n)	Treatment	Time of Measurement*	Synthesis Rate (mgh <sup>-1</sup> )†
I (14)	No halothane	—	14.07 ± 0.76‡
II (6)	Halothane × 1	1 h	11.00 ± 1.29§
III (6)	Halothane × 1	24 h	11.81 ± 1.42§
IV (8)	Halothane × 2	24.5 h	7.07 ± 1.06

\* After start of initial 1-h anesthesia.

† Per 300 mg rat.

‡ Differs from group IV,  $P < 0.01$ .§ Differs from group IV,  $P < 0.05$ .

rate of albumin synthesis to half that of controls (group I).

### Discussion

Our data from the isolated, perfused rat liver show that 1.5% halothane, equivalent to 1.35 MAC, depressed albumin synthesis and transferrin synthesis 43 and 45%. Nearly equivalent suppression of production of these two proteins may be fortuitous or may reflect action on a common pathway of protein synthesis, though at what level is difficult to say. Aune *et al.*,<sup>12</sup> exposing isolated rat hepatocytes to presumably comparable concentrations (2mM) of halothane, found a 65% reduction in uptake of radiocarbon-labeled valine into medium proteins after 2 h. Flaim *et al.* reported a 40% decrease in the rate of incorporation of radiolabeled leucine into albumin by rat livers perfused *in situ* and exposed to 4%, but not to 2%, halothane.<sup>11</sup> A possible explanation for this discrepancy between perfusion studies may be a difference in methodology. In our study, albumin and transferrin production were measured by radial immunodiffusion in a heterologous perfusate. In this way, we avoided the use of radiolabeled amino acids, a technique which may underestimate albumin synthesis in the perfused liver,<sup>17</sup> and possibly obscure differences. Indeed, albumin synthetic rates obtained by us in control perfusions were equivalent to rates accepted for the intact rat. Other reasons for this close approximation to the *in vivo* rate of synthesis may include careful monitoring of perfusate pH and oxygen and carbon dioxide partial pressures and the use of perfusates with hematocrits less than 30%. These measures allow for perfusion over a prolonged period without increases in organ vascular resistance and decreases in bile flow, common monitors of functional integrity of perfused livers.<sup>13</sup> Further evidence for functional integrity of the liver during the perfusion period is provided by the steadily maintained rates of urea, albumin, and transferrin production that were observed. Synthetic rates after more than 4 h of perfusion differed little from those during the first hour. Similarly, Flaim *et al.* noted a steady rate of incorporation of tritiated leucine into secreted albumin during a 2-h perfusion.<sup>11</sup> These

observations may be particularly noteworthy in halothane-treated livers, because they indicate an early effect that is not cumulative.

Our measurements in intact rats by the (<sup>14</sup>C)carbonate technique<sup>25</sup> confirmed the inhibition of albumin synthesis by halothane, but it is interesting that two exposures were required to produce a significant effect in this experimental setting. The (<sup>14</sup>C)carbonate method is based on the concept that "flash" labeling of hepatic protein takes place *via* arginine-6-carbon as albumin is being assembled within the hepatocyte.<sup>25</sup> Incorporation of (<sup>14</sup>C)carbon occurs during the first few minutes after the injection of sodium (<sup>14</sup>C)carbonate, which first enters the arginine-ornithine cycle, labeling both urea and the guanidine (six) carbon of arginine. Albumin synthesis is thus measured "instantaneously" and does not reflect an average estimate over a period of hours. This feature of the method is important in evaluating the effect of halothane exposure, because estimates of the rate of synthesis of export proteins are little affected by changes in concentration of halothane or halothane metabolites that occur after isotope injection, while the <sup>14</sup>C-urea slope is being determined.

Instantaneous measurement in intact animals may help resolve the issue of whether decreased albumin synthesis is due to halothane metabolite accumulation, and it may allow some speculation regarding the double exposure phenomenon. While fluorinated hydrocarbons may be retained to a significant degree for more than 24 h following a single 1-h halothane anesthetic<sup>28</sup> (though this observation has been questioned<sup>29</sup>), albumin synthesis was not significantly depressed 24 h later (group III). Yet, a substantial (nearly 50%) decrease in the rate of synthesis was observed after two exposures to halothane (group IV). It seems unlikely that a second exposure lasting only 30 min could generate enough halothane derivative(s) to play an important role in reducing albumin synthesis, as measured at the end of that 30-min period. A more tenable hypothesis is enhancement by halothane, itself, during a second anesthetic of a previous effect of halothane or possibly of its metabolites on the protein synthesis apparatus. Thus, although a single exposure to halothane was not sufficient in itself to decrease albumin synthesis significantly, this first anesthetic predisposed the synthetic apparatus to marked inhibition immediately following re-exposure. (In fact, a single exposure *may* decrease albumin synthesis; if groups II and III are combined, multiple comparison testing shows their mean of 11.40 mgh<sup>-1</sup> does differ significantly from the control mean of 14.07,  $P < 0.05$ ).

The mechanism of halothane-induced depression of hepatic export protein synthesis is unknown. Reduced splanchnic blood flow during anesthesia has been proposed as a cause of hepatic dysfunction, but, in our

isolated liver preparations, perfusion flow rates did not differ in control and halothane-treated livers. General metabolic depression by halothane also seems unlikely, since bile flow by the perfused livers was maintained during halothane exposure and since urea production, a measure of the activity of the arginine-ornithine cycle, occurred at similar rates in control and halothane-treated livers, perhaps reflecting the presence of sufficient amounts of fatty acids<sup>30</sup> and amino acids<sup>17</sup> in the perfusate plasma. In addition, careful ventilation of intact animals during anesthesia appears to have ruled out hypoxia and hypercarbia as contributing causes.

By whatever mechanism halothane caused decreased albumin and transferrin synthesis, its effect appeared early and remained constant during more than 4 h of perfusion. Further, albumin synthesis in the intact rat was swiftly decreased by a short, second exposure to halothane. This speed and constancy of effect may reflect a direct or indirect action of halothane, itself, on the protein synthesis apparatus, rather than a response due to halothane metabolites accumulating during the course of perfusion. Whether this effect is limited to halothane or is associated with other potent inhalational agents remains to be determined.

Our finding that exposure to clinically relevant partial pressures of halothane depresses albumin (and transferrin) synthesis supports the view that prolonged or repeated exposure to halothane should be employed cautiously, if at all, in patients with compromised protein metabolism.

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