

# Brain Intracellular pH, Blood Flow, and Blood-Brain Barrier Differences with Barbiturate and Halothane Anesthesia in the Cat

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The effects of various depths of barbiturate and halothane anesthesia and different arterial blood carbon dioxide tensions ( $P_{aCO_2}$ ) at uniform levels of anesthesia on brain pH, tissue indicator perfusion, and blood flow were studied in 40 cats. Brain pH was measured using a lipid-soluble, pH-sensitive fluorescent indicator (umbelliferone), and its clearance was determined from the slope of its washout curve. Cerebral blood flow (CBF) was determined from the clearance of intra-arterially injected xenon-133. Values for CBF were higher in the halothane-exposed group, and with deep anesthesia they did not decrease as much as did those seen with pentobarbital (47 vs. 27 ml/100 g/min). The barbiturate brain pH-arterial blood  $P_{aCO_2}$  regression line had a steeper slope than the corresponding halothane line. The two lines crossed at  $P_{aCO_2}$  42 torr and brain pH 7.15. Brain pH was directly related to both  $P_{aCO_2}$  and depth of anesthesia in the halothane-exposed group but only to  $P_{aCO_2}$  in the barbiturate-exposed group. If light halothane anesthesia (0.1 per cent) can be considered almost equivalent to the waking state, then both anesthetics produce relative brain alkalinity. The clearance of the pH indicator was only modestly sensitive to changes in  $P_{aCO_2}$  at anesthetic levels of pentobarbital and halothane. It was not significantly changed by increasing the amount of pentobarbital. However, increasing levels of halothane produced a decrease of more than 50 per cent in its clearance (63 ml/100 g/min at 0.1 per cent vs. 29 ml/100 g/min at 3 per cent). It is concluded that umbelliferone provides a reliable method for the measurement of brain pH and possibly a useful tool for studies of the blood-brain barrier. (Key words: Anesthetics, volatile; halothane. Brain; blood-brain barrier; blood flow; electroencephalography; metabolism; pH. Hypnotics, barbiturates: pentobarbital.)

A pH-SENSITIVE fluorescent indicator for the measurement of intra-cellular brain pH and the pathway of a fat-soluble indicator across the blood-brain barrier (BBB) has been developed. We report here the uses of this new technique, in conjunction with cerebral blood flow (CBF) measurements determined by the intra-arterial injection of xenon-133, for the comparison of brain pH and blood flow in cats at various depths of barbiturate and halothane anesthesia and at

different levels of arterial blood carbon dioxide tensions ( $P_{aCO_2}$ ). The basic technology and instrumentation, which have been described and analyzed in detail previously,<sup>1</sup> are reviewed only briefly here. The indicator used, 7-hydroxycoumarin (umbelliferone), is nontoxic, fat-soluble, and freely diffusible across the BBB. Its molecular and ionic forms are both fluorophors, but with different fluorescent characteristics.<sup>2</sup> Thus, it is possible to create a nomogram relating pH to the ratio of the indicator's 450-nm fluorescent curves from 340-nm 370-nm excitation. Brain pH can then be determined from a ratio analysis of calibrated tissue clearance curves at these wavelengths.

## Methods and Materials

Forty cats weighing between 2.5 and 3.5 kg were anesthetized with pentobarbital, 30 mg/kg, intraperitoneally (20 animals) and halothane, 1.5 per cent (20 animals). Following establishment of anesthesia, cats were prepared by placing catheters into the right femoral artery and vein for monitoring blood pressure, obtaining arterial blood samples, and administering drugs. Arterial blood pressure was measured by a strain-gauge attached to the femoral-artery catheter and displayed on a polygraph. Core body temperature was monitored with a rectal digital thermometer, and the animals were kept at normothermia by the use of a small heating blanket. A tracheostomy was performed and a PE-50 cannula inserted into the right lingual artery so that its tip lay in the carotid artery. The skin, subcutaneous tissue, and muscles were excised over the right parietal area with an electrosurgical unit. Blood losses did not exceed 5 ml. The bone overlying the parietal area was removed with a high-speed air drill. Soft bone wax was placed in the diploë of the bone and the dura was reflected with the aid of an operating microscope over the margins of the craniectomy. The dura was replaced with a thin sheet of plastic (characteristics previously described<sup>1</sup>), which kept the brain moist and prevented surface oxygenation. Three EEG recording electrodes were established over each hemisphere by placing small nickel-plated brass screws through the outer

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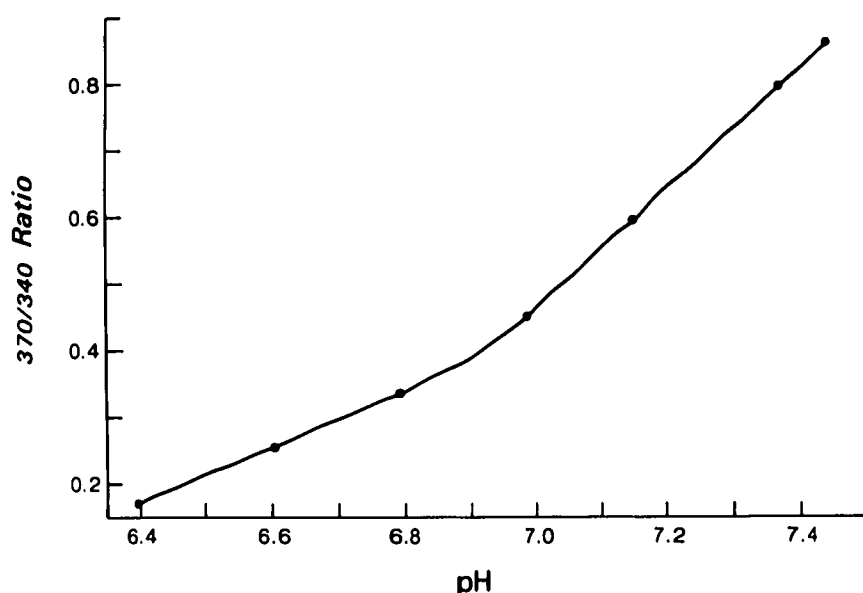


FIG. 1. Excitation of umbelliferone at 370 nm produces a variable, pH-dependent, intensity of fluorescence at 450 nm, but excitation at 340 nm gives an isobestic fluorescence line at 450 nm. A nomogram relating the ratio of these lines to pH permits the determination of pH from the changing fluorescence of the indicator.

layer of bone, after the overlying scalp and muscle had been reflected. The selection of points was uniform and permitted the recording of a four-channel EEG (right and left frontoparietal; right and left frontotemporal). The EEG was recorded on a Grass® Model 78 polygraph. The relative depth of anesthesia was monitored by continuous electroencephalograms (EEG) in each animal. Initial measurements were taken during spontaneous respirations. Pancuronium bromide, 0.15 mg/kg, was then given to abolish respiratory efforts and the animals were ventilated with a Harvard® Model 670 Respirator. Thereafter, cats were assigned to one of four protocols:

**Protocol 1. Barbiturate anesthesia—variable  $P_{aCO_2}$ .** Pentobarbital, 35 mg/kg, was given intraperitoneally and  $P_{aCO_2}$  was changed at 15-min intervals from 40 to 20 to 60 to 40 torr by changing the amount of inspired  $CO_2$ .

**Protocol 2. Halothane anesthesia—variable  $P_{aCO_2}$ .** After surgical preparation, halothane was held constant at 0.8 per cent and  $P_{aCO_2}$  was varied incrementally in a manner identical to that for the barbiturate group. The expired concentration of halothane was monitored with a Beckman Model RB-2 halothane infrared analyzer.

**Protocol 3. Variable barbiturate anesthesia—constant  $P_{aCO_2}$ .** Pentobarbital, 30 mg/kg, was given intraperitoneally, followed by 6 mg/kg intravenously at 15-min intervals until a near-isoelectric EEG was established. The  $P_{aCO_2}$  was held constant at 40 torr.

**Protocol 4. Variable halothane anesthesia—constant  $P_{aCO_2}$ .** After surgical preparation, the halothane concentration was increased at 15-min intervals from

0.1 to 0.5 per cent and then at 0.5 per cent increments to 3.0 per cent or until EEG burst suppression occurred. The  $P_{aCO_2}$  was held constant at 40 torr. It was necessary to administer sodium bicarbonate intravenously during deep halothane anesthesia to maintain a normal arterial blood pH.

For all 40 animals brain pH measurements at death from anoxia were also recorded. Death was produced by inhalation of 100 per cent nitrogen.

Cerebral blood flow was measured by the clearance of a 0.1-ml bolus of physiologic saline solution containing 200  $\mu$ Ci of xenon-133 (total volume of each injectate was 0.3 ml and included 0.2 ml of the solution of umbelliferone). The bolus was delivered into a catheter secured in the right lingual artery. Details of this technique, the isoresponse curve of the detector, and considerations relative to collimation have been described.<sup>3</sup>

A nomogram, derived from the ratio of 450-nm fluorescence from 370-nm and 340-nm excitation, was used for the measurement of brain pH (fig. 1). Each pH value for every animal was determined from these individual clearance curves (fig. 2). Each pH measurement was accompanied by a simultaneous CBF measurement from the clearance of xenon-133 and blood-gas studies.

The techniques, methodology, and instrumentation for these types of studies have been reviewed previously in detail,<sup>1</sup> and are not considered here.

The Student *t* test was used for the analysis of blood flows and indicator clearance rates. The covariance method was used for the analysis of brain pH, as it was necessary to consider in that analysis a second variable, the blood pH.

## Results

The responsivenesses of brain pH, arterial blood pH, clearance of tissue indicator, and CBF to changes in  $P_{aCO_2}$  differed between the cats anesthetized with pentobarbital and those anesthetized with halothane (figs. 3 and 4; table 1). Brain pH values in the two groups were similar at normocapnia and varied directly with change in blood  $P_{aCO_2}$ . Quantitatively, the groups differed, in that the response of brain pH to change in blood  $P_{aCO_2}$  was less in the halothane-exposed group (fig. 3). At all  $CO_2$  levels, the value for CBF was higher in the halothane-exposed group, whereas the indicator clearance rates were less. Both variables varied with  $P_{aCO_2}$ , although the magnitude of change in CBF was much greater. Also, for the halothane-exposed group the slope of the response of CBF to change in  $P_{aCO_2}$  was steeper (fig. 4).

At anesthetic concentrations of halothane (>0.5 per cent), brain pH was significantly increased above that observed at 0.1 per cent and comparable to that observed with pentobarbital anesthesia (table 2). With increasing depths of anesthesia, brain pH significantly decreased in the halothane-exposed group but not in the barbiturate-exposed group. These changes in brain pH were unrelated to any change in blood pH. The groups differed strikingly, in that increasing the depth of halothane anesthesia decreased the rate of indicator clearance by more than 50 per cent, whereas increasing the dose of barbiturate had no measurable effect.

Brain pH values at death from hypoxemia were  $6.77 \pm .04$  and  $6.61 \pm .04$  at halothane, 0.8 and 3 per cent, respectively, and  $6.69 \pm .03$  and  $6.55 \pm .04$  with pentobarbital, 35 and 54 mg/kg, respectively.

Although increasing depths of anesthesia caused depression of the EEG, deep halothane anesthesia did not cause the degree of suppression observed with barbiturate anesthesia (fig. 5).

## Discussion

Umbelliferone is a fat-soluble pH-sensitive fluorescent indicator that is freely diffusible across the BBB and is nontoxic. Both the neutral and the ionic forms are fluorophors.<sup>1</sup> Thus, a nomogram can be constructed from the fluorescent curves at 450 nm from the 370-nm and the 340-nm excitation of this molecule. It is then possible to determine brain pH from the ratio of the emission intensity at these two wavelengths of the indicator clearance curves using appropriate microspectrofluorometric instrumentation and a custom-built filter selector wheel that

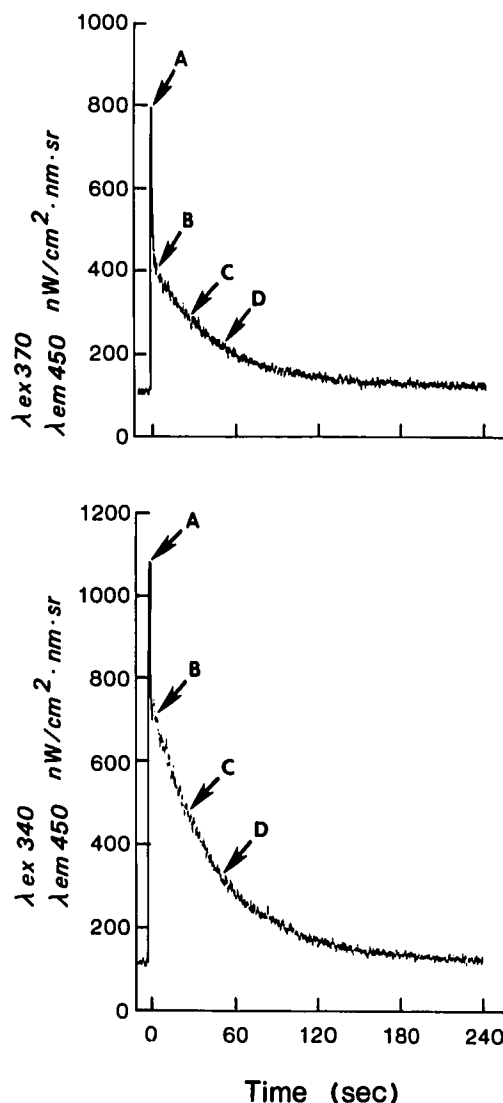


FIG. 2. Typical fluorescent clearance curves of umbelliferone at  $P_{aCO_2}$  40 torr. The equation for the ratio of 370-nm-excited to 340-nm-excited fluorescence in the vascular space (point A) and in the tissue (points B, C, and D) is total fluorescence at 450 nm with 370 nm excitation - 450 nm background fluorescence from NADH-NADPH  $\div$  total fluorescence at 450 nm with 340 nm excitation - 450 nm background fluorescence from NADH-NADPH = 450 nm fluorescence at 370 nm excitation attributable to indicator  $\div$  450 nm fluorescence at 340 nm excitation attributable to indicator. Thus, at point A, the arterial spike, the equation is  $800 - 110 \div 1080 - 110 = 690 \div 970 = 0.711$  (pH 7.280 from nomogram—simultaneous arterial blood sample pH = 7.291); at point B, equation is  $400 - 110 \div 700 - 110 = 290 \div 590 = .491$  (pH 7.032 from nomogram); similar calculations at points C and D produce brain pH measurements of 7.040 and 7.060, respectively. Points B, C, and D are separated from each other by 24-second intervals. Analysis of points further on the clearance curve of umbelliferone are less reliable, as a greater proportion of fluorescence in this portion of the curve is attributable to NADH and NADPH, and therefore, values here are less reliable. Nevertheless, calculations based on these points of the curve appear unchanged from those obtained by use of points B, C, and D.

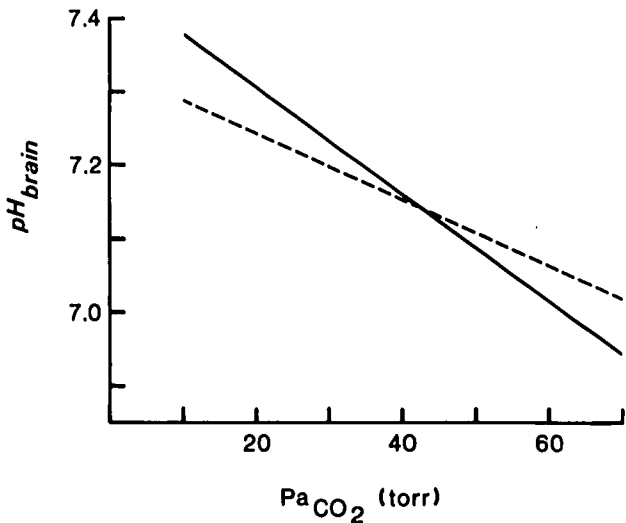


FIG. 3.  $\text{PaCO}_2$ -brain  $\text{pH}$  regression lines. Dashed line = halothane, 0.8 per cent; solid line = pentobarbital, 35 mg/kg.

permits the synchronization of excitation and emission light.<sup>4</sup>

The sources for possible artifacts related to these studies include: alteration of  $pK$  of the indicator as it passes from a hydrophilic to a lipophilic environment, the concentration of the indicator, solvent effect with differential quenching, and changes in the indicator redox potential. These, along with calibration techniques, have been described previously and are not considered here.<sup>1</sup> It is, however, of some importance to note that the calibration data for our fluorescent curves cannot be extrapolated to other spectrofluorometers unless those fluorometers have, at the wavelengths studied, the same relative intensities of excitation. Furthermore, it is also necessary to calibrate the instrumentation frequently, as the nomograms will change

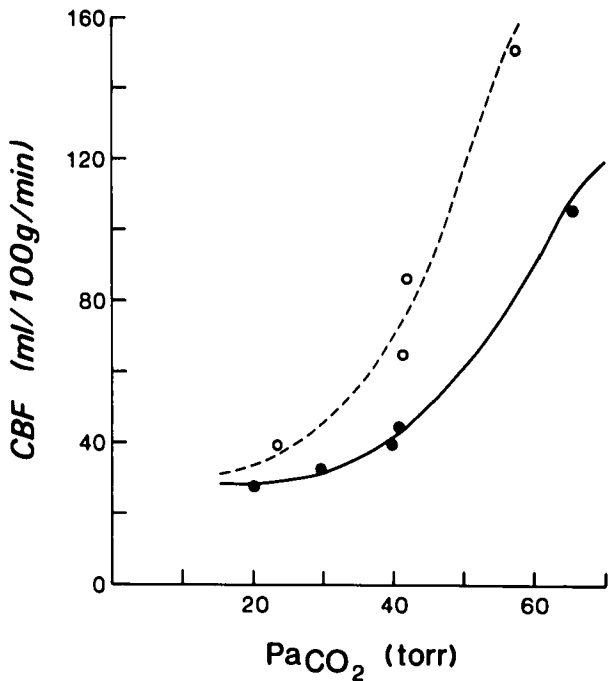


FIG. 4.  $\text{PaCO}_2$ -CBF response curve. Dashed line = halothane, 0.8 per cent; solid line = pentobarbital, 35 mg/kg.

slightly from differential changes in filter related to usage.

Direct measurements of intracellular brain  $\text{pH}$  *in vivo* have not heretofore been possible. All previously published values have been indirectly derived from calculations based upon the distribution of weak acids, such as carbonic acid or 1,3-dimethyloxazolidinedione (DMO), and upon a number of mathematical assumptions.<sup>5,6</sup> Still, the agreement between  $\text{pH}$  values so derived and those measured by indicator fluorescent ratio is quite good. Siesjö<sup>7</sup> calculated normal brain  $\text{pH}$  (unanesthetized)

TABLE 1. Brain  $\text{pH}$  vs. Blood Flow vs. Indicator Clearance with Uniform Levels of Anesthetic Agents and Varied  $\text{PaCO}_2$  Values (Mean  $\pm$  SEM)

	Arterial Blood Samples			Brain $\text{pH}$	Blood Flow (ml/100 g/min) <sup>133</sup> Xe	Indicator Clearance Rate (ml/100 g/min) Umbelliferone
	$\text{P}_{\text{CO}_2}$ (torr)	$\text{P}_{\text{O}_2}$ (torr)	$\text{pH}$			
Barbiturate anesthesia						
Spontaneous	30 $\pm$ 2	84 $\pm$ 5	7.43 $\pm$ 0.02	7.27 $\pm$ 0.03	33 $\pm$ 4	47 $\pm$ 5
Normocapnia	42 $\pm$ 1	118 $\pm$ 13	7.28 $\pm$ 0.01	7.14 $\pm$ 0.03	45 $\pm$ 5	47 $\pm$ 5
Hypocapnia	20 $\pm$ 1	116 $\pm$ 7	7.52 $\pm$ 0.02	7.30 $\pm$ 0.04	27 $\pm$ 1	51 $\pm$ 7
Hypercapnia	66 $\pm$ 4	119 $\pm$ 7	7.02 $\pm$ 0.02	6.98 $\pm$ 0.04	105 $\pm$ 11	56 $\pm$ 6
Normocapnia	40 $\pm$ 3	113 $\pm$ 9	7.25 $\pm$ 0.02	7.13 $\pm$ 0.04	39 $\pm$ 3	56 $\pm$ 6
Halothane anesthesia						
Normocapnia	42 $\pm$ 2	112 $\pm$ 7	7.25 $\pm$ 0.02	7.15 $\pm$ 0.01	87 $\pm$ 12	44 $\pm$ 4
Hypocapnia	23 $\pm$ 1	120 $\pm$ 8	7.53 $\pm$ 0.03	7.24 $\pm$ 0.02	40 $\pm$ 5	29 $\pm$ 4
Hypercapnia	57 $\pm$ 1	113 $\pm$ 9	7.09 $\pm$ 0.02	7.08 $\pm$ 0.02	152 $\pm$ 9	48 $\pm$ 6
Normocapnia	41 $\pm$ 1	112 $\pm$ 4	7.21 $\pm$ 0.02	7.12 $\pm$ 0.02	65 $\pm$ 7	55 $\pm$ 5

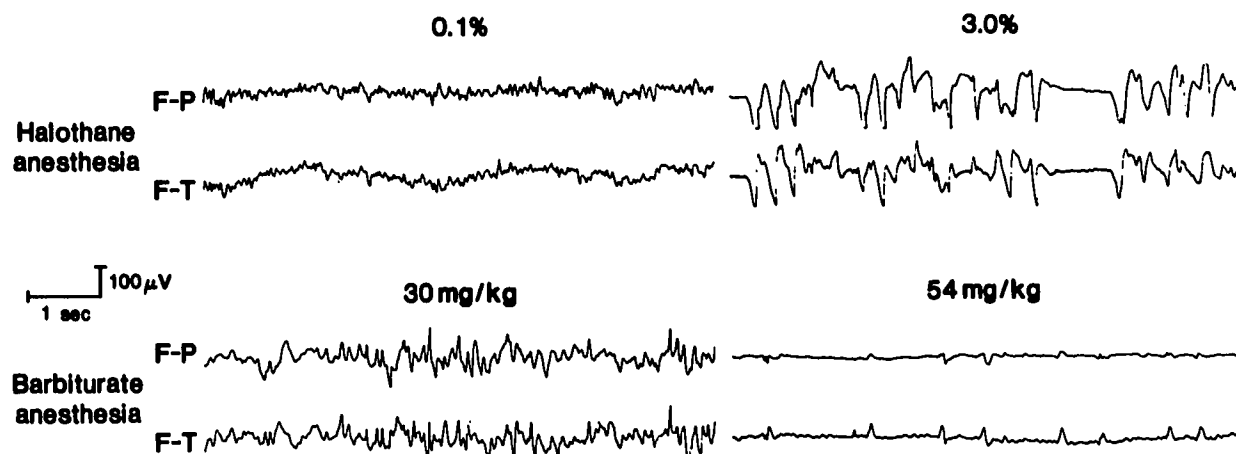


FIG. 5. Typical EEG tracings at various anesthetic levels of halothane and pentobarbital. Deep halothane anesthesia does not achieve the complete paralysis of neuronal function produced by barbiturates.

to be about 7.05. This can be compared with a measured pH of 7.04 in our cats in the presence of halothane, 0.1 per cent. Nilsson and Siesjö<sup>8</sup> and Messeter *et al.*<sup>9</sup> reported a significant alkaline shift in calculated brain pH with induction of halothane anesthesia and a relative brain alkalinity was already present in the barbiturate-anesthetized animals. The magnitude of shift in brain pH that we observed with change in  $P_{aCO_2}$  was similar to that calculated and reported by others.<sup>10,11</sup> Finally, the pH observed at death in our animals, 6.6–6.8, is comparable to that calculated by Siesjö for rats, 6.5 (based upon the magnitude of brain lactate accumulation at death).<sup>7</sup> These favorable comparisons between calculated and measured brain pH values during widely varying circumstances provide further support for the validity of the fluorescent indicator method.

The clearance rates of xenon-133 at various depths

of anesthesia and at different levels of  $P_{aCO_2}$  were exactly what would be expected for normal animals. The various rates of clearance of xenon with different levels of  $P_{aCO_2}$  at uniform levels of anesthesia may be related to extracellular pH, but the exact mechanisms for cerebral vascular reactivity to  $CO_2$  are at present poorly understood. The gradual decrease in CBF with increasing levels of barbiturate anesthesia reflects the expected changes in CBF with decreased cerebral metabolism in this group. In the halothane-exposed group the dose-related decrease in CBF (above halothane, 1.5 per cent) probably relates to a progressive decrease in blood pressure and impaired autoregulation.

A comparison between the clearance rates of xenon-133 and umbelliferone reveals some interesting differences between the clearance rates of an ionized, water-soluble indicator and a non-ionized,

TABLE 2. Brain pH vs. Blood Flow vs. Indicator Clearance, Varied Levels of Anesthetic Agents and Uniform  $P_{aCO_2}$  Values (Mean  $\pm$  SEM)

	Arterial Blood Samples			Brain pH	Blood Flow (ml/100 g/min) <sup>133</sup> Xe	Indicator Clearance Rate (ml/100 g/min) Umbelliferone
	$P_{CO_2}$ (torr)	$P_{O_2}$ (torr)	pH			
Barbiturate anesthesia						
30 mg/kg	39 $\pm$ 1	106 $\pm$ 6	7.27 $\pm$ 0.01	7.11 $\pm$ 0.03	55 $\pm$ 7	49 $\pm$ 5
36 mg/kg	40 $\pm$ 1	109 $\pm$ 6	7.25 $\pm$ 0.02	7.11 $\pm$ 0.03	51 $\pm$ 5	43 $\pm$ 5
42 mg/kg	39 $\pm$ 1	105 $\pm$ 5	7.25 $\pm$ 0.01	7.09 $\pm$ 0.03	38 $\pm$ 5	47 $\pm$ 4
48 mg/kg	41 $\pm$ 1	109 $\pm$ 4	7.23 $\pm$ 0.02	7.08 $\pm$ 0.02	31 $\pm$ 4	47 $\pm$ 4
54 mg/kg	38 $\pm$ 1	112 $\pm$ 6	7.25 $\pm$ 0.01	7.08 $\pm$ 0.02	27 $\pm$ 2	45 $\pm$ 5
Halothane anesthesia						
0.1 per cent	41 $\pm$ 1	127 $\pm$ 5	7.32 $\pm$ 0.02	7.04 $\pm$ 0.02	76 $\pm$ 11	63 $\pm$ 7
0.5 per cent	41 $\pm$ 1	129 $\pm$ 4	7.31 $\pm$ 0.02	7.08 $\pm$ 0.02	80 $\pm$ 10	54 $\pm$ 5
1.0 per cent	40 $\pm$ 1	130 $\pm$ 5	7.31 $\pm$ 0.02	7.15 $\pm$ 0.03	82 $\pm$ 13	51 $\pm$ 7
1.5 per cent	41 $\pm$ 1	137 $\pm$ 5	7.32 $\pm$ 0.03	7.12 $\pm$ 0.03	73 $\pm$ 10	52 $\pm$ 7
2.0 per cent	40 $\pm$ 1	137 $\pm$ 6	7.32 $\pm$ 0.03	7.09 $\pm$ 0.03	68 $\pm$ 10	47 $\pm$ 7
2.5 per cent	40 $\pm$ 1	138 $\pm$ 7	7.32 $\pm$ 0.03	7.08 $\pm$ 0.03	65 $\pm$ 11	39 $\pm$ 5
3.0 per cent	41 $\pm$ 1	145 $\pm$ 9	7.29 $\pm$ 0.02	7.05 $\pm$ 0.03	47 $\pm$ 4	29 $\pm$ 5

fat-soluble indicator. The clearance rate of umbelliferone was much less responsive than that of xenon-133 to alterations in  $P_{aCO_2}$  during moderate barbiturate or halothane anesthesia. This suggests that the transport of the former across the BBB and its partition were not significantly altered by fluctuations in extracellular  $pH$ . The small differences that were seen probably could be explained by changes in CBF.

The relative effects of increasing depths of anesthesia on CBF as determined from the washout of xenon-133 were expected and were similar in the two groups, whereas the clearance rate of umbelliferone was unaffected by barbiturate anesthesia and decreased more than 50 per cent by the higher halothane concentration. An explanation for this striking difference in anesthetic effects is not readily apparent. In comparing the clearance curves of xenon-133 and umbelliferone, it is important to recognize that the xenon clearance curves are derived from the clearance of xenon from the truncated sphere that includes large cortical vessels and white matter.<sup>3</sup> It, therefore, reflects a compartment not visualized with the umbelliferone clearance curves, which are derived from an avascular area of cortex measuring only 106  $\mu m$ . The importance of the artifacts related to the inclusion of the pia-arachnoidal circulation in the xenon-133 clearance curves has recently been reviewed by three authorities on this subject.<sup>12,13</sup>

Intracellular  $pH$  is non-homogeneous, with variable ionic gradients, and therefore, one cannot be certain what portion of the cell was measured in these studies. However, the lipid solubility of the indicator suggests that this was a membranous component. If extracellular  $pH$  is the same as spinal fluid  $pH$ , as disclosed by extracellular electrodes,<sup>14</sup> assumed in formulas used for computing intracellular  $pH$  by mathematical abstractions,<sup>5,8</sup> and suggested from accumulated evidence that extracellular fluid is in a diffusion equilibrium with spinal fluid,<sup>15</sup> then immediately upon leaving the vascular compartment the indicator reflected a  $pH$  that was too acidic to be that of extracellular fluid. Carbon dioxide is known to be freely diffusible across the BBB. Therefore, the non-parallel difference between arterial blood  $pH$  and brain  $pH$  in these studies suggests that this indicator entered an intracellular compartment immediately upon leaving the vascular space.

It is not our purpose here to discuss the possible pathways across the BBB for fat-soluble and non-fat-soluble substances. This subject has recently been reviewed in detail by Rapoport.<sup>16</sup> However, in that the clearance of this fat-soluble indicator

was apparently not affected by barbiturate anesthesia but was changed by deep halothane anesthesia, one is tempted to speculate that deep halothane anesthesia has a direct and possibly deleterious effect on the BBB, whether this be the endothelium or the glial cell adjacent to the basement membrane. Results of both *in vitro*<sup>17</sup> and *in vivo*<sup>18</sup> studies have previously suggested a toxic metabolic effect of halothane at concentrations greater than 2.0 per cent.

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