use of air as part of the respiratory gas mixture. Increased inspired carbon dioxide may be due to the presence of carbon monoxide or the presence of carbon dioxide due to carbon dioxide absorbent exhaustion or leaking valves in a circle system. If 500 ppm (0.05%) carbon monoxide was present in a patient's breathing circuit and was displayed as an increase of either 0.05% nitrogen or 0.05% inspired carbon dioxide, I speculate that this increase would not be distinguishable from innocuous fluctuations of these gases. Therefore, I suggest that, before direct detection of carbon monoxide by mass spectrometry can be used to warn of a patient's exposure to carbon monoxide during anesthetic breakdown, studies must be conducted to show the validity of this technique with clinically relevant concentrations of carbon monoxide.

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Binding of Halothane to Serum Albumin: Relevance to Theories of Narcosis

To the Editor:—The report by Johansson *et al.* provides further insight into the molecular site at which general anesthetics act.[1] The investigators found that halothane quenches the tryptophan fluorescence of bovine serum albumin in a concentration-dependent manner with a dissociation constant of 1.8 mM. They also reported that diethyl ether competes with halothane with a 50% inhibition concentration of 39 mM.

These concentrations surpass those required for anesthesia. At 1.8 mM, halothane equals a partial pressure at 37°C of 0.06 atm (1.8 mM = $(1.8 \times 10^{-6}$ mol/ml) $(2.5436 \times 10^{4}$ ml/mol)/0.75, where 0.75 is the partition coefficient for halothane in Krebs' solution at 37°C.[2] This exceeds the anesthetizing partial pressure of halothane in humans by a factor of 8.[3] Similarly, the partial pressure of ether at 39 mM equals 0.76 atm, assuming a partition coefficient of 13.[4] This exceeds the anesthetizing partial pressure of ether in humans by a factor of 40.[5]

Although Johansson *et al.* performed their studies at 25°C, the above ratios (8 for halothane and 40 for ether) for 37°C will approximate ratios at 25°C because of the counterbalancing changes in solubility and potency of anesthetics with decreasing temperature.[6] Furthermore, the dissociation constant of 1.8 mM for halothane quenching found by Johansson *et al.* is also an order of magnitude greater than the anesthetic potency of halothane measured in animals at lower temperatures: The righting reflex EC_{50} of halothane in tadpoles is approximately 0.1 mM at 20°C.[7] The calculations of partial pressure also assume that the solution used in the experiment was equivalent to an isotonic salt solution. If the albumin added appreciably to the solubility of halothane, this would lower the partial pressure calculated for halothane but not that for ether, whose solubility in blood scarcely differs from that in water.[8,9]

Even allowing for these factors, it appears that the partial pressures applied exceed those that produce anesthesia. If so, can the results provide us with insights into mechanisms of anesthetic action? Does the five-fold difference in the ratios for ether and halothane (8 *vs.* 40) mean that the finding for halothane does not apply equally to all anesthetics, and thus that the tryptophan site is not representative of a relevant anesthetic site of action? Finally, do results obtained at 25°C apply at the higher temperatures sustained by homeotherms?

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In Reply:—Eger and Koblin raise several issues concerning the validity of albumin as a model to study mechanisms of inhalational anesthetic action and the relevance of the concentrations both of halothane and diethyl ether used in our studies. The principal aim of our communication[1] was to describe an alternative and useful approach to determining anesthetic-protein interactions. Albumin was selected to compare the tryptophan fluorescence quenching method with the previous studies on anesthetic binding to this protein, which used ^{19}F-NMR[2] and photoaffinity labeling.[3] Our experiments were performed at 25°C to permit comparison with the earlier studies[2,3] conducted at similar temperatures and because the direct information available[4] suggests that, at least over the temperature range from 5°C to 20°C, volatile general anesthetic binding to protein targets only changes by 20–30%.

The measured dissociation constants of halothane and diethyl ether determined using quenching of tryptophan fluorescence[1] are greater than those required to maintain the anesthetic state in animals. It would be remarkable if the anesthetic binding sites on albumin exactly reproduced the clinical pharmacology of the inhaled general anesthetics. However, the fact that these tryptophan domains bind anesthetics within an order of magnitude of their clinical ED_{50}'s suggests that they may share some characteristics with the physiologically relevant sites. Although this was not the primary goal of the work, this is a satisfactory first approximation, because a tenfold change in affinity only corresponds to a binding energy of 1.4 kcal/mol (comparable to a single hydrogen bond[5]). Our study opens the door methodologically for a molecular description of anesthetic binding sites in proteins: For example, one avenue would be the use of site-directed mutagenesis of the albumin sites to conduct a structure-affinity study with a variety of volatile anesthetic molecules.

In further response to the points made by Eger and Koblin concerning the weakness of the binding and the failure to reproduce the relative order of potencies seen clinically, it should be emphasized that it remains to be established that these structurally diverse molecules act at a single locus. Furthermore, it is far from clear that clinical EC_{50} values must correspond directly to experimental dissociation constants. These questions remain unanswered, because the methodology to directly study volatile anesthetic binding has been unavailable until recently and is under development. Binding studies accounting for anesthetic desolvation and the formation of interactions with protein sites with a focus on molecular structure are needed. Using our approach[1] and other techniques,[2,3] the answers to these questions are forthcoming.

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