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*REVERSIBLE ALTERATION OF THE STRUCTURE OF GLOBULAR PROTEINS BY ANESTHETIC AGENTS**

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Theories of the mechanism of action of general anesthetics have in general assumed either a lipid phase or an aqueous phase to be the site of action.[1] The possibility of interactions between anesthetic gases and proteins has been suggested by scattered observations from several laboratories,[2–5] including our own. Related suggestions are also found in the review of McElroy.[1]

We have now extended our studies on hydrocarbon-protein interactions,[5] where indeed cyclopropane was shown to be effective, to include most of the practical general anesthetics. We employed a polarimetric method to measure these interactions, as described previously.[5] The α_{5460} of a 3 per cent solution of protein in 0.15 *M* NaCl was observed in a 2-cm optical cell, so arranged that on removal from the polarimeter, moist gases could be passed over the solution surface with gentle stirring. Removal of the anesthetic gas from the protein solution was similarly achieved by passing water-saturated nitrogen. In all cases the original rotation was obtained, proving the reversibility of the interactions. With anesthetics boiling above 25°, equilibration was achieved by exposing the solution to the vapor of the pure liquid at 25° in a closed system. Moist N_2 was again used to sweep out the anesthetic vapor and test reversibility. Measurements of optical rotation were made with a Bendix 143a automatic polarimeter with scale expansion to yield a routine precision of ±0.0002° (5460A).

Table 1 shows the changes in optical rotation of (BL) β-lactoglobulin (Pentex, 3× cryst.), and (BPA) bovine plasma albumin (fraction V, Pentex, defatted[6] and deionized[7]), induced by several anesthetic agents and related substances. It is well known that BL undergoes a structural transition between pH 7 and 8. We examined the anesthetic interactions both in the transition (pH 7.75) and at a pH

below the transition (pH 6.70). In all cases BL acquires a less negative rotation in the presence of these gases. Table 1 shows essentially similar results with BPA, at pH 5.5. The same sign of rotation change with all agents implies a qualitative similarity in this group of interactions.

The rotational change effect of divinyl ether with BPA was essentially linear through the pH range 6.5–9.9, slowly decreasing as the pH increases. However, the butane rotational effect increases in the region of pH 7–8, and then levels off in the region of 8–10. This parallels earlier observations[5] on the pH-dependence of butane binding.

The rotatory dispersion of BL was measured in the presence and absence of butane, and while the b_0 parameter (Moffitt-Yang equation, $\lambda_0 = 2120$A, and using data between $\lambda = 6000$ and $\lambda = 3500$A) was unaltered, the value of a_0 increased by about 10 per cent in the presence of butane. If we interpret these results in the conventional way,[8] we are led to the conclusion that butane binding does not alter the helical content but rather affects the arrangement of the side chains of the protein. While these results are suggestive, there is not yet enough evidence to permit generalization on this point.

It is seen in Table 1 that there is a correlation between the potency of a general anesthetic[9] and its effect on the protein structure. Thus, methoxyflurane, chloroform, and halothane, which are efficient anesthetics, produce large structural changes in the protein, while acetylene and nitrous oxide are rather inefficient anesthetic agents and show very small effects on the protein structure. This recalls the earlier work of Clements and Wilson[10] who observed a correlation between the narcotic potency of a compound and its action on surface films of lipids and beef lung lipoproteins on water. We believe that the observations of Clements and Wilson should be re-evaluated to admit that an aqueous interface is not an essential condition for significant interactions.

Our rankings of potency are based on the choice of the standard state to be a hypothetical vapor at a pressure of 1 atm. They therefore yield a different sequence from that obtained by Ferguson.[11] However, our observations appear to be consistent with Ferguson's experimental results in the following ways: (*a*) In an ascending homologous series, both the narcotic potency and the effect of the compounds on protein structure increase. (*b*) Comparing with Ferguson's findings on isonarcotic concentrations, we find that monochlorohydrocarbons do not differ markedly from hydrocarbons in their effect on protein structure, if they are compared with compounds of similar molecular weight. Thus, $(\Delta\alpha)_{\text{butane}} \simeq (\Delta\alpha)_{\text{EtCl}}$ and $(\Delta\alpha)_{\text{propane}} \simeq (\Delta\alpha)_{\text{MeCl}}$; see Table 1. (*c*) Interpolating Ferguson's results between propane and pentane we find butane to have about the same anesthetic potency as diethyl ether, an equivalence which holds in the effects on protein optical rotation (Table 1).

These interactions apparently do not involve covalent bonding, judging from their easy reversibility and from the variety of chemical structures surveyed. The binding of hydrocarbon gases to globular proteins has been suggested, with some justification, to be hydrophobic in nature.[5, 12] It is likely that additional energy components contribute to the binding of some of the anesthetic gases to BL and BSA. Substances like N_2O and even $CHCl_3$ have permanent dipole moments, and their interaction with these proteins may well involve these dipoles. However,

TABLE 1

EFFECTS OF ANESTHETIC GASES ON THE OPTICAL ROTATIONS (α_{5460}) OF β-LACTOGLOBULIN AND BOVINE PLASMA ALBUMIN AT 25°C

Anesthetic agent	Polarizability,[a] cc·10^{24}	Molar vol,[b] l/mole·10^{2}	Press. anesth. agent, mm Hg	% Increase[c] in $-\alpha$, BL pH 6.70	BL pH 7.75	% Increase[c] in $-\alpha$, BPA pH 5.5
1. Methoxyflurane[d]	10	—	30	83	62	62
2. Chloroform	8.5	10.2	199	28	23	46
3. Halothane[e]	9.5	—	300	13.9	13.1	18.7
4. Butane	8.2	12.2	720	8.2	5.9	2.0
5. Ethyl ether	8.9	13.4	537	7.1	5.6	10.1
6. Vinyl ether	8.5	—	645	5.8	4.8	5.0
7. Ethyl chloride	6.5	8.7	715	8.1	3.1	—
8. Propane	6.4	8.6	720	—	3.3	—
9. Methyl chloride	4.6	6.5	715	2.1	3.9	1.8
10. Ethane	4.5	6.4	720	—	1.7	—
11. Xenon	4.0	5.1	710	1.8	1.3	0.9
12. Acetylene	3.7	5.1	720	1.1	0.8	0.23
13. Nitrous oxide	2.8	4.4	720	1.6	1.0	0.14
14. Methane	2.7	4.3	710	—	0.3	—

[a] Calculated from molar refractions.
[b] The van der Waals "b" factor. Values taken from *The Handbook of Chemistry and Physics* (Cleveland, Ohio: The Chemical Rubber Co.), 45th ed.
[c] Calculated as $\frac{\Delta\alpha}{\alpha_{N_2}} \cdot \frac{760}{P} \times 100$. Experimental reproducibility of measured rotations sets an uncertainty of ±0.1%, expressed in the same units as the table entries.
[d] 2,2 Dichlor 1,1-difluoroethyl methyl ether.
[e] Bromochlorotrifluoroethane.

within a homologous series, the effect of binding increases with an increase in the length of the nonpolar chain, as is evident in the methane through butane group and also in methyl and ethyl chlorides. It is also likely that the interaction of anesthetics with proteins will be more discriminating in the matter of size, shape, and polarity than will the simple solubility of these compounds in a bulk lipid phase such as olive oil. Our experiments do suggest some selectivity of binding: the effects of ethyl and methyl chlorides on the rotation of BL at pH 6.70 and at pH 7.75 are reversed. We had also observed earlier that the n-butane interacts more strongly with BL than does isobutane; further, the detailed sequences of effectiveness of these compounds on the rotations of BL and BPA are different.

We have tried to correlate the effect of the bound compound upon the structural change of the protein with its (*a*) polarizability, (*b*) molar volume, and (*c*) molecular weight. There is a rough linear correlation between the effect and the polarizability, as well as with the molar volume of the compounds. However, chloroform, halothane, methoxyflurane, and diethyl ether do not fall into these patterns. It is worth pointing out here that all these compounds boil above 25°C and that equilibration with protein solution was carried out at 25°C, where the vapor pressures of these compounds are substantially below 1 atm. The effects were therefore normalized to 1 atm on the assumption of a simple linear relationship between partial pressure and($\Delta\alpha$). This normalization is probably more valid for BSA than for BL, as shown by Wishnia's studies[4, 12] on pressure-dependent hydrocarbon binding to these proteins. However, since there is a possible systematic error in this extrapolation, it is better to compare the gaseous anesthetics only.[13] Here, good correlations are obtained with molecular properties such as polarizability and molar volume. A slightly poorer correlation is obtained with molecular weight. Thus, it seems that no single one of these molecular parameters is dominantly operative; rather, the interaction and the subsequent effect of these anesthetic agents appears more complex.

Further analysis of these correlations should be deferred until it is possible to compare protein structural changes at isonarcotic concentrations.

The evidence here presented shows the reality of anesthetic-protein interactions, their reversibility, and further shows a correlation of these interactions with anesthetic potency. It finally shows, in model systems, a plausible mechanism (a structural change) by which anesthetic action might be effected, either in a protein or in a lipoprotein structure. It does not, of course, prove that the essential site of these anesthetic agents is necessarily one or more proteins. We submit that the present findings are significant to hypotheses on the mechanism of general anesthesia, as well as in a consideration of side effects.

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[9] Quantitative rankings of the anesthetic protencies of a number of agents can be found in refs. 1 and 10. Unfortunately, no anesthetic potency data exists for the present group of anesthetic agents, obtained on one test organism.

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[11] The fact that a different choice of standard states will give a different pattern of results should not be disconcerting. In Ferguson's choice of standard state the actual anesthetic partial pressure is divided by the vapor pressure of the pure liquid anesthetic agent, while our choice does not refer to the vapor pressure of the pure liquid.

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[13] It would be still better to rank the anesthetics according to the partial pressure required to produce the same change in optical rotation, but this information is more difficult to obtain. The problem is presently being developed in our laboratory.