

INTERACTIONS OF INERT ANESTHETIC GASES WITH PROTEINS

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XENON, an inert monoatomic atmospheric gas with an average atomic weight of 131.3, has been shown by Cullen and Gross¹ to produce anesthesia in man. During some studies on brain saturation and desaturation kinetics with radioactive xenon at Brookhaven National Laboratories several years ago, Featherstone, Pittinger and associates^{2, 3, 4} were able to measure the entrance of the radioactive gas atoms into the brain without difficulty. The exit of the gas could not be determined, however, until the plastic tube (chloroethene polymer $(C_2F_3Cl)_n$) used to conduct the sagittal sinus blood through the counting system was replaced with glass. The difficulty seemed to be that the xenon was associating with the polymeric plastic tube in such a firm way that the gas atoms were not being carried away by the blood, even though the dog was expelling great quantities of xenon with each exhalation.

Although this phenomenon was followed no further at the time, the event led to speculation about the possible associations of xenon

and other anesthetic gases with some of our most common polymeric compounds, the proteins. It is well known that anesthetic gases are generally more soluble in blood than in water, but this increased solubility has been assumed to be due to the lipids in the blood. The oil/water ratios and the blood/gas and water/gas values for several anesthetic agents are listed in table 1. The magnitude of these differences is considerable in some cases, particularly for cyclopropane. In order to determine the extent to which the water and lipid in blood are responsible for the solubility of the gas the following values for the chemical composition of whole blood were used: water, 80 per cent; lipid, 0.56 per cent; protein, 18 per cent; salts, 1 per cent; miscellaneous, 0.44 per cent. The solubilities of cyclopropane at 37 C. in water and in blood are 0.204 and 0.457 ml. gas per milliliter solvent, respectively, and the oil/water ratio of cyclopropane is 35.⁵ A sample calculation is shown below:

Amount of cyclopropane in water of blood:

$$\begin{array}{rcll} \text{water content} & \times & \text{density of} & \text{solubility of gas} \\ \text{of blood} & & \text{blood} & \text{in water} \\ 0.80 & \times & 1.06 & \times 0.204 & = \text{ml. gas in water of 1 ml. blood} \\ & & & & = 0.1730 \text{ ml. cyclopropane in water} \end{array}$$

Similarly, for lipid fraction:

$$\begin{array}{rcll} 0.0056 & \times & 1.06 & \times 0.204 \times 35 & = 0.0424 \text{ ml. cyclopropane in lipid} \\ & & & & \underline{\hspace{1.5cm}} \\ & & & & 0.2154 \text{ ml. due to lipid and water} \end{array}$$

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Blood saturated with cyclopropane at atmospheric pressure contains 0.4570 ml. gas, thus 0.2416 ml. or 53 per cent of cyclopropane in blood is held in associations other than with water and lipid. The 18 per cent protein in the blood could be the factor accounting for

TABLE 1
SOLUBILITIES OF SEVERAL ANESTHETIC
GASES AT 37 C.

	Blood: Gas (ml. gas per ml. solvent)	Water: Gas	Oil-Water Coeffi- cient	Refer- ence
Nitrous oxide	0.466	0.440	3.2	5
Diethyl ether	15.08	15.46	3.2	5
Ethylene	0.140	0.089	14.4	5
Xenon	*	0.085	20.0	6
Cyclopropane	0.457	0.204	34.3	5
Chloroform	7.3	4.6	100.0	7

* No blood: gas data for xenon were available.

the greater part of the solubility of cyclopropane. Similar calculations for ethylene and nitrous oxide show percentages of approximately 41 and 19, respectively, of gas held in associations other than water and lipid.

A search of the literature revealed few references to the possible interactions of protein with inert gases. Kety⁸ commented that the increased solubility of gases in blood *might* be due to proteins. Lesser and co-workers,⁹ in using cyclopropane to measure total body fat, mentioned that protein solutions absorb a great deal of the anesthetic gas and, other than correcting for this in their calculations, ignored the phenomenon. Possati and Faulconer¹⁰ investigated the relationship of hemoglobin concentration and cyclopropane solubility in blood. Their data reveal a linear decrease in cyclopropane solubility as whole blood is diluted with serum. No interaction of the gas with hemoglobin was demonstrated by these authors and the possible interaction of cyclopropane with serum proteins was not considered.

When aqueous solutions of purified serum albumin were used in our laboratory, an increase in the solubility of cyclopropane with increasing protein concentration was observed. The data in table 2 were obtained by using the Van Slyke manometric apparatus and are expressed as the milliliters of gas, corrected to standard temperature and pressure, which will dissolve in 1 ml. solvent (Bunsen coefficient). Further studies will be carried out by using gas chromatography which promises to give more accurate results than can be obtained with the manometric technique. Whole blood

contains approximately 18 per cent total protein, the major portion of which is confined within the erythrocytes (hemoglobin) and lesser amounts in the plasma (albumins and globulins). The calculations presented have shown that 53 per cent of the cyclopropane dissolved in whole blood is not held in solution by the water or lipid fractions present. The percentage of cyclopropane in solution due to albumin at the 18 per cent albumin level, comparable to total blood protein level, is about 40. The major portion of the difference in concentration of cyclopropane in blood from that calculated for water and lipid alone can be accounted for by association of the gas with protein, assuming that gas-protein interaction is not limited to association with albumin, since the major protein in blood is hemoglobin. This is a point which is now under investigation.

Therefore, although ions usually decrease the solubility of gases in solutions, the presence of large multi-charged molecules, such as proteins, seems to increase the number of gas molecules which can be held in solutions. However, not all large charged molecules can increase gas solubility. A water-soluble high molecular weight polymer (Dow Experimental Resin X-2611.1), composed partly of charged quaternary nitrogens, was tested for the ability to increase cyclopropane solubility. It had no measurable effect in this regard. The fact that this resin exists in water as an extended random coil suggests that the more ordered folding of proteins might play a role in their associations with gas molecules.

There has been much speculation in our laboratory as to the mechanism by which inert gases, such as cyclopropane, xenon and nitrous oxide, may interact with protein molecules.

TABLE 2
SOLUBILITY OF CYCLOPROPANE IN SERUM
ALBUMIN SOLUTIONS*

Per Cent Albumin	Bunsen Absorption Coefficient
0	0.281
5	0.291
10	0.344
15	0.361
20	0.387
25	0.419

* In 0.9 per cent NaCl.

Two possible mechanisms are being tested at present.

Since the primary structure of proteins results from the joining together of a series of alpha-amino acids by a peptide linkage involving the carboxyl carbon of one amino acid and the amino group of its neighboring acid, the resulting long chain of amino acids may theoretically assume a number of different secondary and tertiary configurations in solution, depending upon the affinity of different parts of the chain for itself and upon its interaction with the solvent. Some possible configurations have been diagrammatically sketched by Tanford¹¹ and are reproduced in figure 1.

The over-all shape of the molecule may range from a loosely twisted flexible chain (d) to a more tightly packed random coil (c and b) until it acts as a compact rigid sphere (a), impenetrable to solvent molecules. In addition, under certain conditions some proteins are known to exist as rigid rods (e), resulting from the coiling of the amino acid chain into a helical form of very precise dimensions. The existence of the now famous alpha-helix, first proposed by Pauling *et al.*¹² from theoretical considerations, has been demonstrated in a variety of synthetic polypeptides, as well as in hair, muscle and wool. Portions of some of the globular proteins, such as myoglobin, have been found to exist as helices which have twined around to form a more spherical molecule. Other globulins also appear to contain variable proportions of helical conformation interspersed with areas of less ordered structure.

Studies on enzymatic proteins have demonstrated that biological activity is extremely sensitive to changes in secondary and tertiary

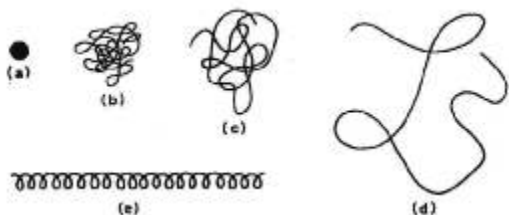


FIG. 1. Schematic representation of possible configurations of an unbranched polymer chain. The fully extended length is the same for each configuration. Reprinted with permission from Albert Neuberger, Symposium on Protein Structure, New York, John Wiley & Sons, Inc., 1958.

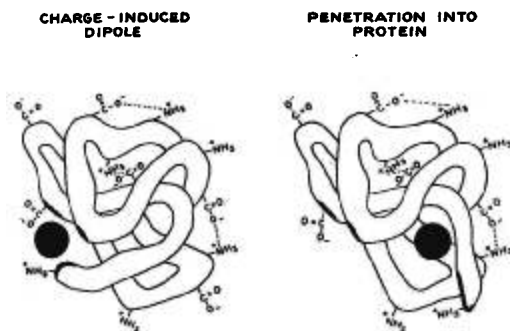


FIG. 2. Schematic representation of two proposed mechanisms of protein-gas interactions.

structure—in other words, to the coiling and folding of the peptide chain.

Two possible mechanisms by which protein molecules may interact with gases are illustrated in figure 2. The first mechanism, on the left, involves the charge on the protein, due to the free amino and carboxyl groups sticking out from its surface, inducing areas of partial negative and positive charge (or a dipole) in the otherwise electrically neutral anesthetic gas molecule, and causing the anesthetic gas molecule to be held at the surface of the protein. With respect to a possible mechanism of action in the living organism, the cell activity might be depressed if this site of association is involved in the action of a protein vital to cell function. The charge-induced dipole mechanism is being tested by measuring the solubility of anesthetic gases in protein solutions in which the charge on the protein is altered but its shape is relatively unchanged. This can be done by altering the pH of a serum albumin solution with and without added salts. In the absence of salt, as the carboxy groups lose their charges below pH 4, the remaining positively charged amino group repel each other and the protein unfolds. But in the presence of added salt, the salt ions shield the positive charges on the protein from repelling one another and the protein stays more or less the same shape as it is at pH 7.

An alternative mechanism which is under investigation is that the anesthetic gas molecule, because it is hydrophobic, penetrates to the interior of the protein in trying to escape from its aqueous environment. It is generally agreed that the residues of the polar amino

acids are on the surface of a protein molecule in aqueous solution and that the nonpolar, hydrophobic residues face inward away from the water. Thus the nonpolar anesthetic gas molecules would prefer the interior environment of the protein if they were able to penetrate into it. The spaces between folds of the protein coils are not vacuums but are normally filled with solvent molecules, inorganic ions and nitrogen molecules. Since the common anesthetics are larger molecules than nitrogen it might be expected that in attempting to penetrate into the protein interior, the anesthetic molecule may cause some disruption and unfolding of the protein coils. A possible result of such an occurrence is depicted on the right side of figure 2. By partially opening up the protein molecule while penetrating into it, the anesthetic molecule has twisted the hypothetical active site mentioned earlier out of the correct steric relationship for biological activity. If anesthetic gases do indeed cause such changes in molecular volume, the occurrence would be reflected in changes in viscosity of gas-saturated protein solutions. Studies of this nature are being planned.

The relation, if any, between the helical nature of some proteins and their possible ability to increase gas solubility is a third phase of our investigation. The helical content of several proteins is now known and this quantity may be increased by the addition of certain organic solvents, such as chlorethanol, which change the electrical nature of the solution and cause more of the protein to assume a helical configuration. Studies will be carried out on the solubilities of anesthetic gases in protein solutions containing varying amounts of chlorethanol in order to determine whether there is any correlation of helical content and solubility. In addition, several synthetic polyamino acids are known to be rigid helical rods in solutions of one pH and change to random coils at different pH. Measurement of gas solubility in acidic solutions of polyglutamic acid and basic solutions of polylysine, in which these two peptides are helices, are being planned and will be compared to gas solubilities in basic solutions of polyglutamic and acidic solutions of polylysine, in which the polymers are random coils.

It is interesting to note that Pauling,¹³ in

commenting on the possible role of protein-inert gas interactions in anesthesia, as proposed in this paper, suggests that the interaction of protein side-chains with water and anesthetic gas molecules causes the formation of microcrystal hydrates *in vivo*. Formation of such hydrates, Pauling argues, would interfere with the normal electrical oscillations of the nervous system and lead to anesthesia. Pauling's hypothesis is far more specific than any previously proposed. It is theoretically consistent with our data on the solubility of gases in the presence of proteins, and it offers encouragement for pharmacologists and anesthesiologists to consider further the role of specific proteins and proteins in specific locations in the production of general anesthesia.

Since the beginning of the twentieth century, when Meyer and Overton showed the correlation between the solubility of narcotic agents in lipoids and their anesthetic potency, the attention of investigators in the field of anesthesia has been largely directed to some type of lipid mechanism. Although the limitations of the lipid theory have long been recognized, many individuals have not seriously considered other phases of biological systems as involved in anesthesia. The purpose of this paper is to turn the attention of investigators to other components of biological systems. Preliminary work in our laboratory indicates that there is a demonstrable interaction of "inert" anesthetic gases with proteins. Perhaps functionally important associations with these compounds or other non-lipoid biological materials may also be demonstrated.

SUMMARY

Calculations have been presented to demonstrate that not all of the cyclopropane, ethylene and nitrous oxide present in a blood sample saturated with gas at atmospheric pressure can be accounted for by association with the lipid and water in blood. Preliminary studies in our laboratory have indicated that increasing solubility of cyclopropane results when the gas is equilibrated with solutions of increasing protein concentration. The quantity of cyclopropane dissolved in an 18 per cent human serum albumin solution (in 0.9 per cent NaCl) is nearly equal to the cyclopro-

pane dissolved in whole blood, excluding that portion of gas associated with the lipid and water fractions of blood.

A brief description is given of experiments being conducted in our laboratories to elucidate the mechanism of interaction of inert anesthetic gases with proteins in aqueous solution.

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REFERENCES

1. Cullen, S. C., and Gross, E. G.: Anesthetic properties of xenon in animals and human beings, with additional observations on krypton, *Science* **113**: 580, 1951.
2. Featherstone, R. M., Steinfield, W. Gross, E. G., and Pittinger, C. B.: Distribution of anesthetic gas xenon in dog tissues as determined with radioactive xenon, *J. Pharmacol. Exper. Ther.* **106**: 468, 1952.
3. Pittinger, C. B., Featherstone, R. M., Gross, E. G., Stickley, E. E., and Levy, L.: Xenon concentration changes in brain and other body tissues of the dog during inhalation of gas, *J. Pharmacol. Exp. Ther.* **110**: 458, 1954.
4. Pittinger, C. B., Conn, H. L., Featherstone, R. M., Stickley, E., Levy, L., and Cullen, S. C.: Observations on kinetics of transfer of

- xenon and chloroform between blood and brain in dog, *ANESTHESIOLOGY* **17**: 523, 1956.
5. Orcutt, F. S., and Seevers, M. H.: Solubility coefficients of cyclopropane for water, oils and human blood, *J. Pharmacol. Exp. Ther.* **59**: 206, 1937.
6. Lawrence, J. H., Loomis, W. F., Tobias, C. A., and Turpin, F. H.: Preliminary observations on narcotic effect of xenon with review of values for solubilities of gases in water and oils, *J. Physiol.* **105**: 197, 1946.
7. Evans, F. T., and Gray, C.: *General Anaesthesia*, Vol. 1, London, Butterworth & Co., Ltd., 1959, p. 308.
8. Kety, S.: Theory and applications of exchange of inert gas at the lungs and tissues, *Pharmacol. Rev.* **3**: 1, 1951.
9. Lesser, G., Blumberg, A., and Steele, J.: Measurement of total body fat in living rats by absorption of cyclopropane, *Amer. J. Physiol.* **169**: 545, 1952.
10. Possati, S., and Faulconer, A.: Effects of concentration of hemoglobin on solubility of cyclopropane in human blood, *Anesth. Analg.* **37**: 338, 1958.
11. Tanford, C.: *Configuration of Globular Proteins in Aqueous Solution and Its Dependence of pH*, Symposium on Protein Structure, New York, John Wiley & Sons, Inc., 1958, p. 35.
12. Pauling, L., Corey, R., and Branson, H.: Structure of proteins: two hydrogen-bonded helical configurations of polypeptide chain, *Proc. Nat. Acad. Sci. U.S.A.* **37**:205, 1951.
13. Pauling, L.: Molecular theory of general anesthesia, Science, in press.

RUPTURED STOMACH There have been no reported cases of rupture of the stomach in adults due to oxygen administration. Relaxation of the superior esophageal sphincter probably occurs with sleep and anesthesia, especially during the course of surgery and the immediate postoperative period. The volume at which the stomach will rupture has been reported at 4 liters. The stomach generally ruptures along the lesser curvature near the cardia. The patients present the clinical picture of abdominal catastrophe with acute gastric dilatation, muscular rigidity, absent peri-

stalsis, and pain. Severe shock rapidly occurs and subcutaneous emphysema has been noted in some patients. Confirmatory evidence can be established by roentgenogram. Immediate laparotomy is necessary when the diagnosis has been made. Three cases in which rupture of the stomach occurred during nasal oxygen administration are presented. (*Walstad, E. M., and Conklin, W. S.: Rupture of the Normal Stomach After Therapeutic Oxygen Administration, Report of Three Cases, New Engl. J. Med.* **264**: 1201 (June 8) 1961.)