2. E. Schraga, H. A. Lardy, R. C. Nordlie, and D. O. Foster, *J. Biol. Chem.* **238**, 3188 (1963).
3. D. L. DiPietro, *J. Biol. Chem.* **239**, 4051 (1964).
4. W. Z. Hassid and S. Abraham, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. 3, p. 34. Academic Press, New York, 1957.
5. B. Bloom, *Anal. Biochem.* **3**, 85 (1962).
6. E. Schram, *in* "Organic Scintillation Detectors," p. 75. Elsevier, Amsterdam, 1963.
7. B. Bloom, F. Eisenberg, and D. Stetten, *J. Biol. Chem.* **215**, 461 (1955).
8. T. Moriwaki and B. R. Landau, *Endocrinology* **72**, 134 (1963).

Further Studies on the Solubilities of Xenon and Cyclopropane in Blood and Protein Solutions

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SUMMARY

The solubility of xenon and krypton in whole blood and plasma has been measured chromatographically and shown to vary from species to species. Some physical-chemical parameters of another nonhydrogen bonding gas–protein system (cyclopropane–serum albumin) have been studied, and no changes in the degree of protein–gas interaction could be observed with changes in pH, temperature, or ionic strength of the solutions.

The interactions of certain nonhydrogen bonding gases with bovine proteins have been demonstrated by Featherstone *et al.* (1) and Muehlbaecher *et al.* (2) and discussed by them in a review published in 1963 (3). Calculations based on the data presented in these papers show that the molar ratio of gas to protein (Table 1) varied when aqueous buffered solutions of different proteins were equilibrated with several pure gases at 37° at atmospheric pressure. Approximately four molecules of cyclopropane associated with each molecule of hemoglobin and serum albumin. Under the same conditions, hemoglobin associated with only one molecule of xenon. These three systems were selected for further study.

Chromatographically pure xenon, krypton, and cyclopropane were used. The several times recrystallized bovine serum albumin is claimed by the manufacturer (Pentex Corporation, Kankakee, Illinois) to be electrophoretically homogeneous. Whole, heparinized blood and protein solutions were saturated at atmospheric pressure in a Van Slyke manometric apparatus, thermostated at either 37° or 25°; aliquots of the saturated solutions were removed anaerobically for analysis in a Beckman GC-2A gas chromatograph. The latter was fitted with a reaction chamber of a Fisher clinical gas partitioner, which permitted direct analysis of the aqueous samples. Analyses of xenon and krypton were carried out by using a 1-foot molecular sieve 13× (42/60 mesh) column, maintained at 220°. A 4-foot column of di-*n*-decyl phthalate (30% on C-22 Firebrick, 42/60 mesh) at 100° was used for cyclopropane measurements. Known volumes of pure gas were

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TABLE 1
Associations of gases at atmospheric pressure with bovine proteins in aqueous solution at 37°

Protein	Molecular weight	Moles of gas/mole of protein			
		Nitrous oxide	Cyclopropane	Xenon	Krypton
Hemoglobin	68,000	1.6	4.1	0.9	0.3
Serum albumin	67,000	0.8	3.8	0.5	0.3
β-globulins	93,000[a]	0.9	0.5	—	—
γ-globulins	169,000	—	1.0	—	—

[a] Human.

used to calibrate the instrument, and the area under the appropriate curve on the chromatograph recorder was assumed to be proportional to the gas content of the sample. Each value is the average of at least three replicate experiments and is expressed as the Bunsen coefficient.[2]

TABLE 2
The effect of ionic strength on the solubility of cyclopropane in aqueous solutions of bovine serum albumin

Solution	Ionic strength	
	Bunsen coefficient at 37°	
	0.013	0.130
Buffer	0.213 ± 0.009	0.207 ± 0.005
3.7% albumin	0.262 ± 0.005	0.245 ± 0.049
7.4% albumin	0.300 ± 0.025	0.300 ± 0.012
11.1% albumin	0.334 ± 0.039	0.338 ± 0.028
14.8% albumin	0.383 ± 0.031	0.380 ± 0.038
18.5% albumin	0.400 ± 0.027	0.415 ± 0.004

The results of studies in which the pH and the ionic strength of bovine serum albumin solutions were altered independently, indicate that the solubility of cyclopropane in solutions of serum albumin is not sensitive to differences in ionic strength (Table 2) or changes in pH between 4.7 and 8.4. The Bunsen coefficients at 37° for 7.4% serum albumin solutions at pH 4.7, 5.7, 6.3, 6.8, 7.4, 7.7, 7.9, and 8.4 were 0.269, 0.277, 0.266, 0.255, 0.260, 0.277, 0.269, and 0.273, respectively. Thus alterations in charge on the protein molecule do not affect the cyclopropane–albumin interaction, within the pH range studied. It has been shown that no major alterations in molecular volume of the albumin molecule occur at these pH's (4) although changes in conformation have been reported above pH 7 (5).

Temperature studies on the solubility of cyclopropane in serum albumin (Fig. 1) indicate that lowering the temperature has

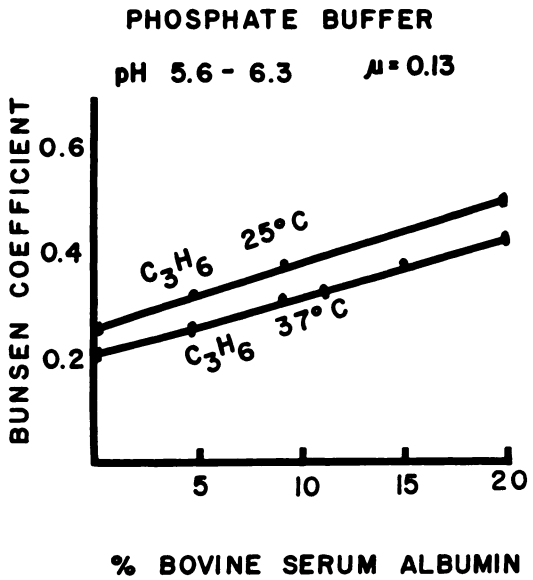

FIG. 1. *The effect of temperature on the solubility of cyclopropane in solutions of bovine serum albumin*

[2] The Bunsen coefficient is defined as milliliters of gas, corrected to standard temperature and pressure, per milliliter of solution, when the pressure of the saturating gas has been corrected to 760 mm mercury.

no effect on the degree of gas–protein interaction. The solubility of the gas in the aqueous protein solution was increased by lowering the temperature of the system, but all the increase can be accounted for by the increased solubility of the gas in the aqueous portion of the solution; the entire solubility curve is shifted upward, without change in slope. The ratio of gas solubility in 18.5% albumin to its solubility in buffer is 2 at both temperatures studied, and therefore the number of gas molecules associated with each protein molecule is unchanged. These observations are similar to the results reported by Wishnia (6) for hydrocarbon solubility in protein solutions.

Before further studies with purified hemoglobin and the metabolically inert gases were initiated, it seemed of sufficient interest to determine the solubilities of xenon and krypton in whole blood, as such values were not readily available in the literature. The values obtained, shown in Table 3, indicate that there is a difference in the solubility of xenon in the whole blood of animals of different species due to differences in the uptake of gas by the erythrocytes. Canine, mouse, and hamster erythrocytes take up approximately three times as much xenon as does an equal volume of plasma, while the cell:plasma ratio for guinea pig, bovine, and human blood is approximately 2. Although the plasma protein concentrations of these species varied somewhat, the total amount of gas dissolved in the plasma did not differ significantly among the animals tested, with the possible exception of the dog. It is also evident that xenon and krypton behave very much like cyclopropane, ethylene, and chloroform in that they are more soluble in whole blood than in aqueous salt solutions (1). In saturated whole blood, 45% more xenon and 51% more krypton are dissolved than can be accounted for by the solubilities of these gases in water and lipid of blood. Similar values were obtained for cyclopropane and ethylene (2). It has not yet been determined whether the species differences of xenon solubility are caused by differences in the lipoprotein content of the erythrocyte stromas of the various species or by the possible qualitative and quantitative differences in cellular hemoglobin content. In view of the greater solubility of anesthetic gases such as ethylene, and cyclopropane (7), and of xenon and krypton in the erythrocytes (Table 3), compared to plasma, it may be postulated that hemoglobin plays

TABLE 3
Distribution of xenon and krypton in whole blood

Sample	Bunsen coefficients at 37°			Ratio cells: plasma	Plasma protein concentration (%)
	Per ml whole blood	Per ml plasma	Per ml cells[a]		
Xenon[b]					
Guinea pig	0.118 ± 0.008	0.094 ± 0.016	0.147	1.6	6.9 ± 0.3
Bovine	0.148 ± 0.007	0.101 ± 0.005	0.211	2.1	7.7 ± 0.4
Human	0.149 ± 0.006	0.102 ± 0.008	0.206	2.0	7.3 ± 0.2
Canine	0.152 ± 0.001	0.080 ± 0.004	0.245	3.1	7.2 ± 0.3
Mouse	0.182 ± 0.017	0.099 ± 0.020	0.290	3.0	8.6 ± 0.1
Hamster	0.192 ± 0.007	0.099 ± 0.013	0.292	3.0	8.1
Krypton[c]					
Human	0.075 ± 0.005	0.048 ± 0.002	0.105	2.2	8.3 ± 0.7

[a] Obtained by difference.
[b] Bunsen coefficient at 37° of 0.13 ionic strength phosphate buffer = 0.079.
[c] Bunsen coefficient at 37° of 0.13 ionic strength phosphate buffer = 0.056.

an important role in the transport of anesthetic gases in the blood.

The literature concerned with the solubility of different anesthetic gases in tissues and blood of different species under uniform conditions using a single end point for the definition of "anesthesia" is extremely limited. Yet most theories of anesthesia, past and present, are necessarily based upon the composite values of different species and different degrees of anesthesia. The data presented here seem to indicate that for a single agent, xenon, one cannot extrapolate, from one species to another, even such a basic value as blood-gas concentration. The studies on the cyclopropane–serum albumin interaction indicate that protein–gas associations are complex systems and not as easily explained as might be hoped.

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REFERENCES

1. R. M. Featherstone, C. A. Muehlbaecher, F. L. DeBon and J. A. Forsaith, *Anesthesiology* **22**, 977 (1961).
2. C. A. Muehlbaecher, F. L. DeBon and R. M. Featherstone, *Intern. Anesthesiol. Clinics* **1**, 937 (1963).
3. R. M. Featherstone and C. A. Muehlbaecher, *Pharmacol. Rev.* **15**, 97 (1963).
4. C. Tanford, J. Buzzell, D. Rands and S. Swanson, *J. Am. Chem. Soc.* **77**, 6421 (1955).
5. W. Leonard, K. Vijai and J. Foster, *J. Biol. Chem* **238**, 1984 (1963).
6. A. Wishnia, *Proc. Natl. Acad. Sci. U.S.* **48**, 2200 (1962).
7. J. Adriani, "The Chemistry of Anesthesia," p. 402. Thomas, Springfield, Illinois, 1959.

Enhancement of 5-Iododeoxyuridine Incorporation into DNA of Cat Tissues *in Vivo* by Inhibition of Uridine-Deoxyuridine Phosphorylase

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SUMMARY

When ^{131}I-iododeoxyuridine (IUdR) (31 μmoles/kg) was given to cats, the *in vivo* incorporation into DNA of bone marrow, intestine, and spleen was increased 3- to 7-fold by deoxyglucosylthymine (TdG) given at a ratio IUdR:TdG = 1:2, and 5- to 14-fold at a ratio 1:5.

Contrary to the extensive incorporation of IUdR[1] into the DNA of bacteria, phages, and cells in tissue culture, extremely low incorporation is seen in living animals (1–5). This is due to the very rapid degradation of 5-halogenated deoxyuridines in the animal body (1, 2, 6), limiting the therapeutic effectiveness of these compounds. Despite repeated attempts to inhibit this process (2, 4, 7–9), no increase in incorporation produced in this way has been reported as yet. The following results show that it is really possible to improve IUdR incorporation into the DNA of animal tis-

[1] Abbreviations: DNA = deoxyribonucleic acid; IUdR = 5-iododeoxyuridine; TdG = 1(3)-[2-deoxy-D-*arabino*-hexopyranosyl]-thymine (deoxyglucosylthymine).