The data confirm our previous conclusion[6] that T2-specific RNA is not a stable catalyst.

* Aided by a Fulbright Fellowship and a Scholarship of the C.N.R. (Italy).
† Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

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*THE SOLUBILITY OF HYDROCARBON GASES IN PROTEIN SOLUTIONS**, †

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Communicated by Walter H. Stockmayer, October 10, 1962

In 1959, Kauzmann[1] called attention to the role of the so-called hydrophobic interactions (i.e, the effects of nonpolar groups on the structure of water pointed out by Frank and Evans[2]) in stabilizing folded protein structures. The measure of this stabilization is the free energy of transfer of an alkyl residue on a peptide chain from an aqueous environment to the interior of the protein. Several model systems have been considered[1, 3] in which the hydrocarbon gases figure prominently.

It would seem that the transfer of alkanes from water to protein (their relative solubilities in water and protein solutions) resembles the desired process very closely: One may imagine a hypothetical bond forming to the coil and splitting in the folded structure. If the gas were bound to specific sites (which, as will be seen, is probably not the case), the cratic terms in entropy and free energy could easily be eliminated by choosing the standard state of the gas in water as unit mole-fraction and calculating the binding constant. When, however, the gas is merely constrained to limited portions of the surface and of the interior of the protein, the choice is less obvious, but, as a consequence, the data obtained may afford further insight into

the problem of protein structure. The determination of the enthalpy of transfer is relatively unambiguous.

Accordingly, the solubilities, as functions of temperature, of butane, propane, and, for BSA, ethane in water and in solutions of bovine serum albumin (BSA), hemoglobin (Hb), lysozyme, and sodium lauryl sulfate (SLS), were studied.

Materials and Methods.—All gas-solubility apparatus is a compromise between sensitivity and precision, and protein solutions pose additional problems. The apparatus used, which has the general flavor of a Warburg or Barcroft manometer, was built with the following considerations in mind: All glass high-vacuum (0.1 μ) design (to prevent contamination of the gases), small (10 ml) solution volumes, smaller (5–10 ml) gas volumes, large gas-liquid interface, weighing-in of solutions, outgassing *in situ* without loss of solvent or harm to the protein, no contact of mercury of solution with grease, no contact of protein with mercury, minimal exposure of gas to grease, absolute pressure measurement, and precision-bore manometers. The entire apparatus is thermostatted to ± 0.01°C in a large well-stirred bath.

The initial and final gas pressures and volumes, together with the compressibilities,[4] are used to compute the molal solubilities of the gases. Since for the concentrations and pressures used (< 1 atmos) no deviations from Henry's Law were observed (implying that the proteins have either a very large number of binding sites or, in fact, no fixed sites at all), the data presented are normalized to one atmosphere. The reproducibility of replicate samples, $\pm 1\%$, while not outstanding, is sufficient for the conclusions that will be drawn.

The gases, Phillips Research Grade Hydrocarbons, were used without further purification. Crystalline BSA (Pentex Lot BX3), deionized on a Dowex-1 OH^- Dowex-50 H^+ column and analyzed by the method of Dole,[5] contained no fatty acid contaminant. The proteins that could be assayed were not damaged by the procedure: Human Hb (gift of Arthur Samuels) examined at the end of a run, showed no change in the oxy Hb or reduced Hb spectra; the activity of Worthington twice-crystallized lysozyme against *Micrococcus lysodeikticus* was unchanged. SLS samples of high purity by surface tension criteria[6] were generous gifts of Arno Cahn of Lever Brothers and James Barnhurst of Colgate-Palmolive Company.

Results and Discussion.—In Figure 1 is shown the solubility of butane in 5 per cent BSA, 5 per cent Hb, and water. The curve for 1.8 per cent SLS is double the BSA curve. The curve for 10 per cent lysozyme lies only about 5 per cent above the water curve. It is clear that the effects are quite large and protein-specific.

The solubility increments per 10^3 gm of solute may easily be computed; these are given as Arrhenius plots in Figures 2 and 3.

The first point to note is the (approximately) threefold decrease of solubility of propane with respect to butane. For BSA and ethane, a further three- to four-fold decrease has been observed. Such effects are expected in nonpolar liquids:[7, 8] The ideal solubility of these gases, given by Raoult's Law, depends inversely on their vapor pres-

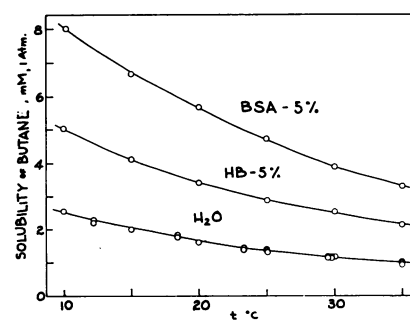

FIG. 1.—Solubility of butane, millimoles per 1,000 gm solution, 1 atmosphere.

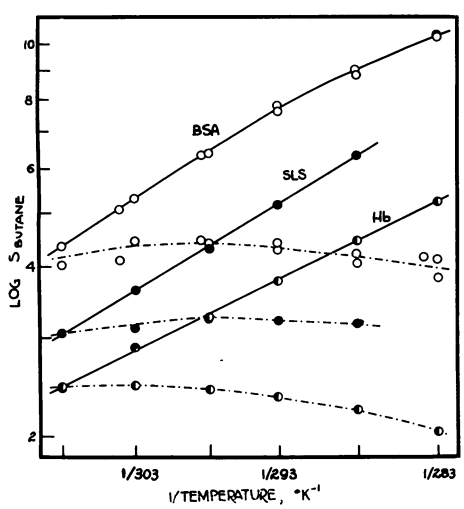

FIG. 2.—Heats of solution, butane.

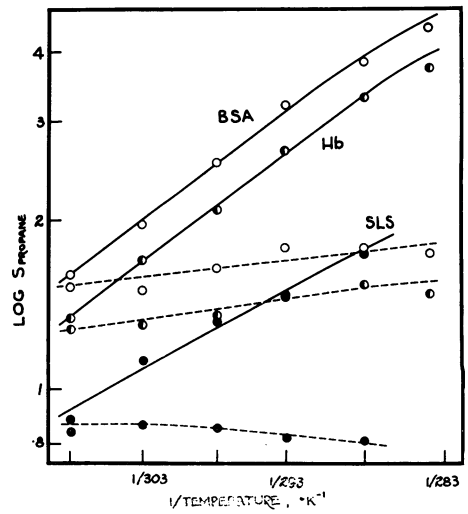

FIG. 3.—Heats of solution, propane.

Open circles, *BSA*; half-filled circles, *Hb*; filled circles, *SLS*. Solid lines, solubility increment, millimoles per 10^3 gm solute at 1 atmosphere. Dashed lines, ratio of solubility in the solute to solubility in water. N.B., values for *BSA* and *Hb* to be multiplied by 10, *SLS* by 100.

sures at the temperatures in question. The solubilities in water are remarkable in that, insofar as there is any difference, the order is ethane > propane > butane.[9, 10] Further, the behavior of BSA and Hb parallels that of SLS. The solubility increments for the effective solutes must, therefore, represent a direct interaction between the gases and the proteins (or the micelles). The small effect of lysozyme may represent a true solubility or a slight solvent effect.

The second point, the key observation, is shown in the dashed lines: The ratio of solubility in protein to solubility in water is almost independent of temperature (the curvature of the lines arises largely from the curvature of the water line). At 25°, the ΔH of transfer of butane or propane from water to the interior of SLS micelles is approximately zero. The ΔH of transfer of butane from water to BSA is also close to zero at 25°, while for Hb the transfer is slightly endothermic (+0.8 kcal). Because of the curvature, these values change with temperature. The transfer of propane from H_2O to BSA and to Hb is slightly exothermic (−0.5 and −1.0 kcal, respectively, at 25°). For ethane and BSA, ΔH appears more negative, but this system needs further study. I wish to emphasize that these results confirm the prediction[1] that the hydrophobic interactions are primarily entropic in nature, with only small enthalpic contributions.

The final point concerns the detailed picture of the interactions. Since the molal solubilities are known, after choosing standard states one may readily obtain numbers for the ΔF and ΔS of transfer of the alkanes from water to solute. This procedure may still be meaningful for SLS micelles, and the unitary[1] parameters are given herewith: At 25°, propane $\Delta F_u = -4.3$ kcal, $\Delta S_u = +16$ e.u.; butane $\Delta F_u = 5.1$ kcal, $\Delta S_u = +17$ e.u. Corresponding values for the transfer from water to the pure liquefied gases are −5.1 kcal, +23 e.u., −5.9 kcal, +23 e.u.;[1] for transfer to 2-propanol, −4.1 kcal and −5.0 kcal.[8] The microscopic micellar systems are

probably not far from the macroscopic limit,[11] and a straightforward gas-in-liquid solubility is indicated.

For the proteins, such a calculation is more likely to obscure than to illumine the rather close relationships suggested by the similar enthalpies. The "mole-fraction" solubility of butane and propane in BSA is much greater than the ideal solubility, and the model of protein-as-liquid-drop is not particularly attractive. The question is rather why the solubility of butane in BSA and Hb is $^1/_8$ and $^1/_{12}$ that in SLS, when on a weight basis it should be more like a third or a half. It cannot be a surface phenomenon, since lysozyme, which has a greater surface to mass ratio, has so little effect—especially as lysozyme may be almost all surface.[12] The hypothesis is that the gas molecules are differential probes for clusters of hydrophobic residues. There is no particular handle to the hydrocarbon gases, so that one would not expect their behavior toward SLS to differ fundamentally from their behavior toward similar groups on proteins. Other things equal, the relative solubilities of butane and propane should be the same in different systems. In fact, however,

$$(S_{butane}/S_{propane})_{protein}/(S_{butane}/S_{propane})_{SLS}$$

is less than unity, being 0.7 for BSA and 0.5 for Hb, indicating that butane, at least, may be large enough to show an effect of size. The fact that the much larger isoöctane is bound to BSA only after massive binding of SLS[13] supports this notion.

Two types of experiment suggest themselves immediately: (1) modification of the protein (e.g., the acid expansion of BSA); and (2) application of a wider variety of probes (methane to pentane, the branched alkanes, alkenes) to a protein of known structure (myoglobin). These experiments are being performed.

Summary.—A study of the solubility of butane, propane, and ethane in solutions of BSA, Hb, lysozyme, and SLS has established three points. (1) The usually large (lysozyme is an exception) solubility increment represents a direct interaction between the gas and the other solute, best understood as solubility in a nonpolar material. (2) The ΔH of transfer of gas from water to the macro solute is close to zero, confirming the prediction by Kauzmann and others that the contribution of hydrophobic interactions toward stabilizing folded protein structures arises primarily from entropy changes. (3) Results obtained on BSA with propane, butane, and, by other workers, isoöctane suggest that the investigation of the relative and absolute solubilities of a graded series of alkanes may provide an estimate of the number and size of hydrophobic clusters in a given protein.

* Supported from funds on NSF Grant No. G 13973 and USPHS Grant No. RG 8121(C1).
† Presented in part at 142d meeting, American Chemical Society, September 1962.

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ACKNOWLEDGMENT

V. Bargmann informs me that the basic theory of his article "Remarks on a Hilbert space of analytic functions," these PROCEEDINGS, vol. 48 (1962), p. 199, is due actually to I. E. Segal and was presented in one of a course of lectures given at the 1960 Summer Seminar in Applied Mathematics, Boulder, Colorado (sponsored by the American Mathematical Society), and in Chapter 6 of the duplicated notes issued concurrently (or see the forthcoming book based thereon, entitled *Mathematical Problems of Relativistic Physics*). Mr. Bargmann much regrets the oversight, which arose from exceptional circumstances, represented by the omission of reference to this work.

WILLIAM FELLER

ERRATUM

In the article entitled "Selection for geotaxis in monomorphic and polymorphic populations of *Drosophila pseudoobscura*," by Theodosius Dobzhansky and Boris Spassky, which appeared on pages 1704–1712 of volume 48, the last two lines in the paragraph below Table 2 on page 1709 should read as follows: "In this population, the selection has evidently favored a gene pool, which made the AR/AR and CH/CH homokaryotypes semilethal, and thus achieved a population in which almost two thirds of the individuals were the heterokaryotypes AR/CH. Populations of this sort are known in some species of *Drosophila* also in nature."