The Solubilities of Seven Gases in Olive Oil With Reference to Theories of Transport Through the Cell Membrane[1]

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Abstract

The solubilities of He, Ne, Ar, N_2, O_2, CO and CO_2 in olive oil have been determined in the temperature range 24–56 C. The gas solubility apparatus was a considerably improved version of the one reported by Morrison and Billett and gave a precision, depending on the gas solvent system of ±0.2% to ±1.0%. Plots of log L, the Ostwald coefficient, and log α, the Bunsen coefficient, against 1/TK were linear and showed a small temperature dependence for CO_2. The enthalpy and entropy changes corresponding to the solution process were calculated. The partition coefficients of the gases between olive oil and water were calculated using reported values for the solubility of the gases in water. These results have been used to test and extend the Meyer-Overton theory of transport through the cell membrane.

Introduction

THE PRINCIPAL OBJECTIVE of this study was to test and extend the work of Collander et al. (1–3) who demonstrated that there is an approximately linear relationship between the olive oil water partition coefficients and the permeability constants of all membranes for a large number of organic compounds. They found that the permeability constants of several types of cells to a variety of nonelectrolytes can be predicted by the relation:

$$P = bK^m/M^n \text{ (Collender equation)}$$

where P is the permeability constant, K is the partition coefficient between a lipid solvent and water, M is the molecular weight of the solute, m is a constant dependent on the lipid solvent, and b and n are empirical constants. The molecular weight of the solutes studied varies only slightly compared with variations in the partition coefficient. Following Collander, we have therefore made use of the simplifying assumption that the P–K relationship can be studied by plotting log P against log K. The effect of the molecular weight could be seen on these graphs.

The gases considered in this study are lower in molecular weight and higher in permeability constant than most of the substances known to obey the Collander equation. The establishment of reliable values of the lipid-water partition coefficients for these solutes is important in several ways: (a) The measurements for CO, CO_2 and O_2, for which permeability constants of human red blood cells have been measured (4) allow testing of the validity of the equation over a range considerably in excess to that covered by previously available results. (b) The results on He, Ne, Ar and N_2 allow, depending on the validity of the Collander equation, prediction of the permeability of cell membranes of these gases. This is of practical interest for N_2 and Ar which are present in air, and also for He and Ne which have both real and potential uses as constituents of artificial atmospheres. The results will permit further testing of the equation when direct methods of measuring the permeability of these gases are developed. (c) Since the solubility of gases in olive oil is almost exactly the same as in various fats, e.g., human, dog and rat (5), the results are of general physiological application.

Experimental Procedures

The method was developed from that of Morrison and Billett (6). The basis of the method is the attainment of equilibrium between the gas and the solvent by allowing the latter to drip into and flow through a known volume of the gas. Our apparatus incorporated improvements introduced by Clever et al. (7) and included some additional modifications. The apparatus was housed in an air thermostat. Temperature control was effected using a Hallikainen Thermodyne proportional controller. The design of the air thermostat to achieve very uniform temperature was rather similar to that of Cook (8). The temperature was measured to ±0.003 C with a platinum resistance thermometer calibrated at the triple point of water and at the benzoic acid point using benzoic acids cells calibrated by the National Bureau of Standards. The maximum variation of temperature with time was found to be ±0.04 C over 8 hr, which is a longer period of time than the longest solubility determination. In most cases the variation during a determination was less than ±0.03 C. The temperature gradient throughout the length of the apparatus was somewhat less than 0.1 C. The error introduced by these temperature variations was sufficiently low to be insignificant for the systems studied. The apparatus is shown in Fig. 1.

The procedure can be conveniently considered in two parts: (a) Degassing: The degassing technique has been described in a separate publication (9). The principle involves the use of an all-glass circulating pump to continually spray the solvent into an evacuated chamber. Provisions for heating, stirring and monitoring of the pressure are incorporated. After degassing, the solvent is transferred without any contact with the atmosphere, into a storage spiral inside the air thermostat (Fig. 1). (b) Solubility determination: The degassed solvent is allowed to stand in the storage spiral for a minimum period of 2 hr (usually overnight, in practice) to reach the temperature of the thermostat. The apparatus is then filled with the gas under study to a pressure of just less than 1 atm. The solvent is allowed to drip into the absorption spiral A until the 10 ml burets B are filled to about a third of their capacity. The solvent flow is stopped and the drainage timed. From 30–60 min after the end of the first solvent flow, levels of the solvent in the 10 ml burets and the mercury in the 50 ml buret C are measured with a cathetometer. The pressure is adjusted to atmospheric and fixed by closing the tap in the ex-

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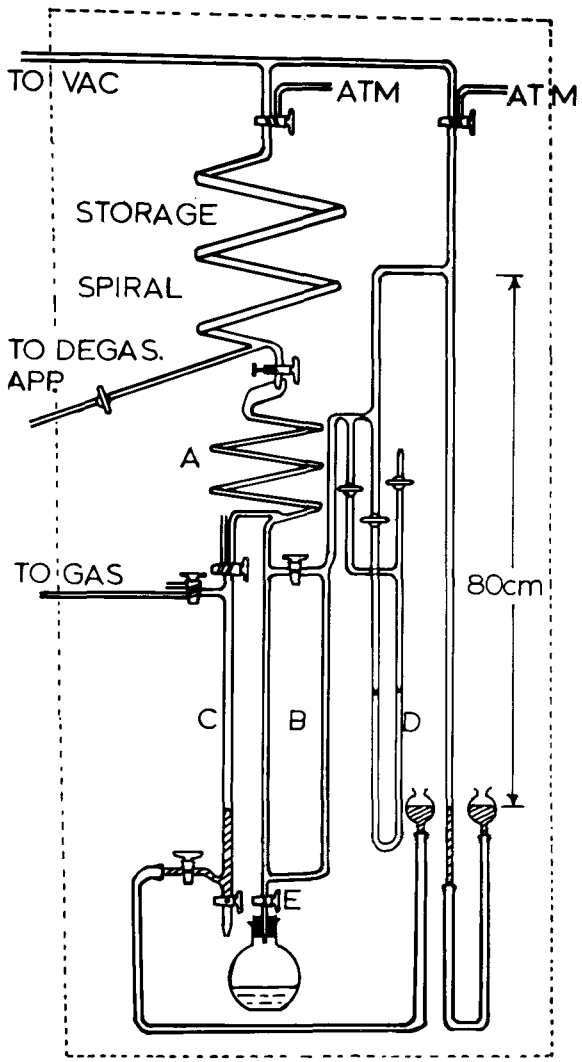

FIG. 1. Solubility apparatus.

ternal limb of the silicone oil manometer D. Solvent is allowed to drip into the apparatus at a rate previously determined to be in the range at which complete saturation occurs. The levels in the silicone oil manometer and the 10 ml burets are kept as nearly equal as possible during the process of solution so that the pressure remain close to the original atmospheric pressure. Excess solvent is run from the apparatus through the stopcock E at approximately the input rate. The solvent is collected in a tared flask. After a sufficient volume of gas has been dissolved, the flow is stopped. The pressure throughout the apparatus is adjusted to the initial value and the levels in the 10 ml burets and the 50 ml buret are measured. From these observations and the weight and density of the solvent, it is a simple matter to calculate the solubility.

Materials

All the gases used were research grade (The Matheson Co., Inc., East Rutherford, N. J.), except for carbon monoxide (Linde Co., Tonawanda, N. Y.). Olive oil from two sources was used: Fisher U.S.P., Fisher Scientific Co., and Nutritional Biochemicals Corp. The oil was characterized by density measurements, which were also used in the calculations. The relationship

$$d_t(\text{g cm}^{-3}) = 0.915_2 - 0.00046_8 t(\text{C})$$

TABLE I

Solubilities of Seven Gases in Olive Oil at Atmospheric Pressure

Gas	Temperature (C)	Bunsen Coeff. (α)	Mole fraction ($\times 10^3$)
He	24.69	0.0160_7	0.701
	34.71	0.0157_0	0.688
	44.83	0.0149_8	0.661
	54.78	0.0145_9	0.647
Ne	24.52	0.0195_7	0.854
	24.82	0.0198_0	0.864
	34.75	0.0194_4	0.853
	35.00	0.0192_2	0.843
	45.50	0.0188_2	0.830
	54.85	0.0184_4	0.817
Ar	24.76	0.136_8	5.96
	34.69	0.133_2	5.84
	45.21	0.129_1	5.70
	54.36	0.126_3	5.60
O_2	25.11	0.1162	5.07
	35.05	0.1171	5.10
	45.38	0.1185	5.22
	54.78	0.1195	5.29
N_2	24.78	0.0674_8	2.94
	24.89	0.0688_0	3.00
	34.80	0.0657_7	2.88
	45.05	0.0644_5	2.84
	54.56	0.0636_1	2.82
CO	24.79	0.0835_2	3.64
	25.06	0.0843_3	3.68
	35.00	0.0863_7	3.79
	35.04	0.0874_9	3.84
	44.44	0.0874_3	3.85
	54.78	0.0904_4	4.01
CO_2	24.85	1.295	56.5
	34.76	1.105	48.5
	45.35	0.960_5	42.1
	54.79	0.868_3	38.5

was established to $\pm 0.1\%$ between 24 and 56 C. No difference in density or solubility of gases was observed in the oil from different sources. The free fatty acid concentration was different but was low in both instances: 0.58% for the Fisher product and 0.30% for that from N.B.C. Earlier workers (10) have also produced evidence for the view that olive oils from various sources show similar solubility characteristics for gases.

The average molecular weight needed for the calculation of the mole fraction solubility was determined from the nuclear magnetic resonance spectrum (11). The value found was 884 g mole^{-1}, with experimental limits of error of $\pm 5\%$.

Results

The solubilities were calculated in three forms (see Battino and Clever (12) for a complete discussion of gas solubility units): (a) the Ostwald coefficient, $L = V_g/V_l$, where V_g is the volume of gas absorbed by the volume of solvent, V_l, all measured at the same temperature; (b) the Bunsen coefficient, $\alpha = 273.15\ L/T$, where T is the temperature in K; and (c) the mole fraction, $X = L\,\tilde{V}_1^\circ/RT$, where $\tilde{V}_1^\circ$ is the molar volume of the solvent and X is calculated for a gas partial pressure of one atmosphere,

The results are presented in Table I. Plots of log α were linear to $\pm 0.2\%$ in the case of O_2, $\pm 0.5\%$ in

TABLE II

Heats and Entropies of Solution at 25 C

Gas	ΔH (cal mole^{-1})	ΔS (X exptl) (cal mole^{-1} deg^{-1})	ΔS ($X = 10^{-4}$) (cal mole^{-1} deg^{-1})
He	−546	−1.75	+2.12
Ne	−327	−1.05	+3.22
Ar	−421	−1.35	+6.77
N_2	−360	−1.15	+5.57
O_2	+300	+0.97	+8.76
CO	+562	+1.80	+8.96
CO_2	−2,150	−8.02	+4.57

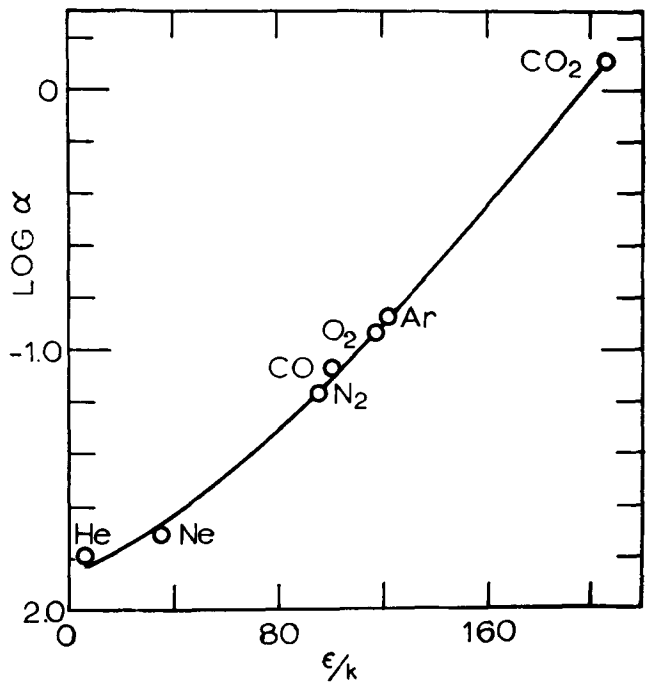

FIG. 2. Logarithm of the Bunsen coefficient vs. ϵ/k for several gases in olive oil.

the case of He and CO, and ±1.0% in all other cases. Only in the case of CO_2 is the deviation from linearity significantly greater than the expected precision. The results for the more soluble CO_2 were better represented by an equation including the second power of the temperature.

The heats and entropies of solution calculated from the variation of log X with temperature are shown in Table II. The heat of solution was assumed to be constant over the temperature range of the solubilities. $\Delta S(X \text{ exptl})$ was calculated for transferring gas at 1 atm to the solution at the experimental mole fraction solubility of the gas, and $\Delta S(X = 10^{-4})$ was calculated for transferring gas at 1 atm to a mole fraction of 10^{-4}. See Hildebrand and Scott (23) for a discussion of entropy of solution as related to gases. The partition coefficients of the gases between olive oil and water at 25 C were calculated from our results and literature values for gas solubilities in water are shown in Table III. Finally, results obtained by previous investigators were compared with those of the present study and this comparison is summarized in Table IV.

Discussion

The connection of the gas solubilities with the energy parameter, ϵ/k, of the Lennard-Jones 6–12 potential for the gases, and the surface tension of the solvent, were examined for olive oil. The correlation found with ϵ/k is illustrated in Fig. 2. A smooth

TABLE III

Partition Coefficient for Seven Gases Between Olive Oil and Water at 25 C

Gas	Olive oil Bunsen coeff.	Water Bunsen coeff.	Partition coeff. (P)
He	0.0161	0.00847 (13)	1.90
Ne	0.0196	0.0102 (13)	1.92
Ar	0.137	0.0307 (14)	4.47
O_2	0.116	0.0263 (15)	4.41
N_2	0.0681	0.0146 (15)	4.66
CO	0.084	0.0214 (16)	3.94
CO_2	1.295	0.745 (17)	1.74

TABLE IV

Comparison of Solubility (Bunsen Coefficient) In Olive Oil With Earlier Work

Gas	Temp. (C)	Earlier results	This work
He	38.0	0.0148 (18)	0.0154
Ne	37.6	0.0193 (19)	0.0191
Ar	38.0	0.1395 (18)	0.132
O_2	38.0	0.102 (20)	0.117
	25.3	0.102 (21)	0.116
N_2	38.0	0.06673 (18)	0.0655
	37.4	0.06092 (19)	0.0655
	25.2	0.628 (21)	0.068
CO		(No comparable results)	
CO_2	50.0	0.839 (31)	0.913
	45.0	1.015[a] (5)	0.961
	30.0	1.063 (31)	1.182
	25.0	1.357[a] (5)	1.295

[a] Other results (22) on related substances (cottonseed oil and some fats) indicate that Y and P results (5) may be high.

curve connects all points. If straight lines were drawn they would exclude either He and Ne or He and CO_2. There appears to be no reason to favor one or the other. The correlation with solvent surface tension (12,24) was reasonable though not highly precise.

These relationships are theoretically interesting and they are also useful for the estimation of unknown solubilities. Their significance should not be overestimated, however. The theory that the dissolving of a gas can be regarded as a two-stage mechanism is fairly well established (12,24). One stage involves the transfer of molecules from the gas phase to a condensed state and this is conditioned by the intermolecular force relationships in the two states—hence the correlation with ϵ/k. The other stage involves the insertion of the solute molecules into so-called cavities in the solvent. This will involve forces dependent on the cohesive energy in the liquid—hence, the surface tension. This is presumably a secondary correlation, i.e., surface tension and solubility are both related to the internal cohesive energy of the solvent.

The partition coefficients (Table III) for three of the gases (CO, CO_2 and O_2) were used to extend the correlation found previously between partition coefficients and permeability constants at 25 C of human erythrocytes as reported in reference 4 and 25–27 for the substances (listed in order of increasing permeability) erythritol, malonamide, thiourea, glycerol, lactamide, methylurea, ethylene glycol, acetamide, propionamide, urea, water, methanol, ethanol, CO_2, CO and O_2. Permeability data is not available for the other gases reported in this study. For the above-mentioned substances (excluding the gases and ethylene glycol and glycerol) we plotted the log of the permeability constant, P, against the log of the olive oil-water partition coefficient, K, and the following equation was fitted to the data by a least squares analysis:

$$\log P = 1.47 \log K - 0.81$$

The points for ethylene glycol and glycerol were not used because there is evidence (28,29) that they enter the red blood cell through specific sites not available to other penetrating molecules. The malonamide value was determined in the laboratory of one of the authors (Danforth) and allowed conversion of the other published results to cgs units so that the units of P in the above equation are cm sec^{-1}. Of the compounds other than the three gases, the P values for only 2 (erythritol and urea) of 13 fell more than 1.5 log units above or below the fitted line. The three gases, however, fell 1.8–2.8 log units below the line. The permeability coefficients for O_2, CO and CO_2 are

thus roughly one hundred times smaller than predicted by the equation. A straight line satisfying the points corresponding to the gases as well as the other substances is not impossible in view of the generally high scatter we found. However, the smaller size of the gas molecules would lead one to expect their permeability coefficients would be higher, not lower (as found), than predicted by the above equation.

We are inclined to the view that the observed deviations require some modification of the theory. The relationship described by the Collander equation has been related to a physical model in which penetration of the cell membrane involves diffusion through a thin homogeneous lipid layer. The prediction of a linear relation between the partition coefficient and the permeability constant assumes, in effect, that diffusion through the interior of the membrane is so much slower than diffusion across the membrane surfaces that the penetrating substance is essentially at equilibrium across each of the two surface layers. Davson and Danielli (28) have given reasons for believing that this assumption may not be valid for rapid-penetrating substances. Thus, while we believe that the Collander equation requires some modification, the results would not necessarily be inconsistent with the model on which the equation was based. It has been noted (30) that biological activities are related in part to cell permeability and increase with the lipid-water partition coefficient up to an apparently optimal value and then decrease.

Addition of our results to earlier ones still leaves the question in doubt. Measurements of additional constants in the range above 0.01 cm sec^{-1} would be valuable. In the meantime our research is aimed at improving the model system so that partition coefficients more closely related to those of the actual cell membrane can be evaluated.

On a practical level, assuming that a smooth relationship exists between the permeability constant and the partition coefficient, it is reasonable to make estimates of the permeability of erythrocytes to He, Ne, Ar and N_2 using our data. It would be most interesting to compare these predicted values with direct measurements.

ACKNOWLEDGMENT

This work was supported by the Public Health Service grants GM 12071 and GM 14710-01.

REFERENCES

1. Wartiovaara, V., and R. Collander, "Permeabilitätstheorien" (Protoplasmatologia II/c8d), Springer-Verlag, Wien, 1960.
2. Collander, R., and H. Bärlund, Acta Bot. Fennica *11*, 1 (1933).
3. Collander, R., Physiol. Plantarum 7, 420 (1954).
4. Roughton, F. J. W., Progr. Biophys. Chem. *9*, 97 (1959).
5. Yeh, S.-Y., and R. E. Peterson, J. Pharm. Sci. *52*, 453 (1963).
6. Morrison, T. J., and F. Billett, J. Chem. Soc., 2033 (1948).
7. Clever, H. L., R. Battino, J. H. Saylor and P. M. Gross, J. Phys. Chem. *61*, 1078 (1957).
8. Cook, M. W., "U.S. Atomic Energy Commission, UCRL-2459," 1954 (Ph.D. Thesis).
9. Battino, R., and F. D. Evans, Anal. Chem. *38*, 1627 (1966).
10. Nussbaum, E., and J. B. Hursh, J. Phys. Chem. *62*, 8 (1958).
11. Johnson, L. F., and J. N. Shoolery, Anal. Chem. *34*, 1136 (1962).
12. Battino, R., and H. L. Clever, Chem. Rev. *66*, 395 (1966).
13. Morrison, T. J., and N. B. Johnstone, J. Chem. Soc., 3441 (1954).
14. Douglas, E., J. Phys. Chem. *68*, 169 (1964); Ibid. *69*, 1608 (1965).
15. Morrison, T. J., and F. Billett, J. Chem. Soc., 3819 (1952).
16. O'Brien, H. R., and W. L. Parker, J. Biol. Chem. *L*, 289 (1922).
17. Yeh, S.-Y, and R. E. Peterson, J. Pharm. Sci. *53*, 822 (1964).
18. Behnke, A. R., and O. D. Yarbrough, U.S. Naval Med. Bull. *36*, 542 (1938).
19. Ikels, K.-G., Defense Documentation Center Report SAM-TDR-64-1; SAM-TDR-64-28.
20. Rodnight, R., Biochem. J. *57*, 661 (1954).
21. Davidson, D., P. Eggleton and P. Foggie, Quart. J. Exptl. Physiol. *37*, 91 (1952).
22. Nichols, G., Science *126*, 1244 (1957).
23. Hildebrand, J. H., and R. L. Scott, "Regular Solutions," Prentice-Hall, Inc., Englewood Cliffs, 1962, chapter 4.
24. Uhlig, H. H., J. Phys. Chem. *41*, 1215 (1937).
25. Paganelli, C. V., and A. K. Solomon, J. Gen. Physiol. *41*, 259 (1957).
26. Jacobs, N. H., J. Cellular Comp. Physiol. *4*, 161 (1934).
27. Höber, R., and S. L. Orskov, Arch. Ges. Physiol. *231*, 599 (1933).
28. Davson, H., and J. F. Danielli, "The Permeability of Natural Membranes," 2nd ed., Cambridge Univ. Press, New York, 1952.
29. Bowyer, F., Intern. Rev. Cytol. *6*, 469 (1957).
30. Hansch, C., and T. Fujita, J. Amer. Chem. Soc. *86*, 1616 (1964).
31. Tomoto, N., Bull. Fac. Eng. Miyazaki Univ., *39*, (1958); Tomoto, N., and K. Kusano, Yukagaku *16*, 108 (1967).

[Received January 3, 1968]