INFLUENCE OF THE DIELECTRIC CONSTANT IN BIOCHEMICAL SYSTEMS

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INTRODUCTION

The dielectric constant presumably varies widely in biochemical systems. Urea, the amino acids and peptides, the proteins and the phospholipoids are known to increase the dielectric constant of even so polar a solvent as water. Fats, on the other hand, have low dielectric constants. All are important constituents of the body. The dielectric constant of different tissues may thus be expected to vary widely, depending upon the electrical properties of the molecular components.

Strong electrolytes are constituents of all biological systems, and so the dielectric properties of cells and tissues cannot be measured by existing methods. Instead it has been necessary to purify constituents of such systems until their conductivity was low, and then measure the dielectric constants of their solutions. Such measurements have now been made in a number of laboratories on a large number of amino acids and peptides, and the results, with widely different methods, are extremely concordant. Measurements upon amino acids and peptides, purified or synthesized by one or another of my collaborators, have been reported to you in the preceding paper by Wyman, and we can assume this knowledge in the following discussion. The measurements on egg albumin and hemoglobin made by Errera in Belgium have essentially been confirmed in our laboratory by Oncley, who is extending them to other proteins and to solvents of other dielectric constants.

The impetus to all this work came, of course, from the investigations of Debye. It had long been known that amino acids, peptides, and proteins were amphoteric electrolytes. In the same year in which Debye explained the behavior of strong electrolytes in terms of interionic forces, Bjerrum (3)—developing an idea that had previously been advanced by Bredig (8) and Adams (2)—demonstrated that amino acids, in the isoelectric condition, were not uncharged molecules, as had previously been assumed, but were what had been called zwitterions, and what for lack of a better

name, we shall call dipolar ions.[1] Although these neutral molecules have no net charge, and therefore do not move with the electric current, they bear equal numbers of positively and negatively charged groups on their surface. These dissociated groups give rise to electrostatic forces, which influence solvent molecules, other ions, and dipolar ions. It was to be hoped, therefore, that the development for dipolar ions of Debye's electrostatic force theory would offer fresh insight into the behavior of these important molecules. My colleagues, Scatchard and Kirkwood (34, 54), have both contributed to this aspect of the problem, and I shall leave to them its presentation, retaining for myself the task of describing the phenomena, whenever possible in quantitative terms, that need to be understood if we are to have an insight into systems containing many components,—some ions, some dipolar ions, and some uncharged molecules.

I. CHANGE IN FREE ENERGY WITH CHANGE IN SOLVENT

Born and Fajans in 1920 considered the change in free energy involved in the transfer of ions from an infinitely dilute gas to an infinitely dilute aqueous solution, and formulated an equation which may be written

$$\bar{F} - \bar{F}^{\text{gas}} = \frac{N\epsilon^2 z^2}{2b}\left(\frac{1}{D_0} - 1\right) \tag{1}$$

in which ϵ is the elementary charge of the electron, z the valence of the ion, b its radius, and N Avogadro's number. If the transfer is from water to some medium other than a vacuum, which has the dielectric constant D, this expression becomes

$$\bar{F} - \bar{F}^{0} = \frac{N\epsilon^2 z^2}{2b}\left(\frac{1}{D} - \frac{1}{D_0}\right) \tag{2}$$

Debye and McAulay (22) employed this equation in their study of mixed solvents. They followed Born in regarding the ions as electrical spheres of radius b. The solution outside this radius b was treated as continuous and of uniform dielectric constant.

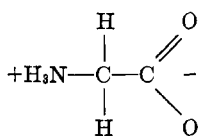
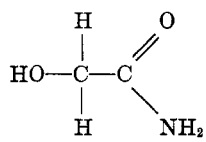
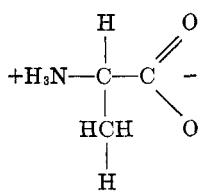
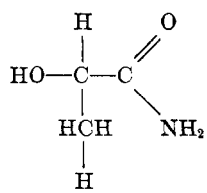
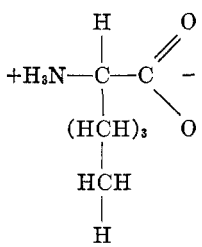
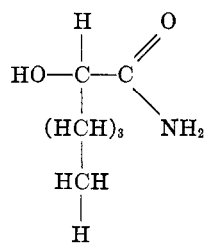
The extension of this equation to the case of dipolar ions, although it has had a preliminary theoretical treatment (34, 54), has heretofore lacked an adequate experimental background. In recent studies we have estimated that part of the change in free energy due to electrostatic forces by comparing amino acids and peptides with comparable uncharged molecules (41, 42). Thus the logarithm of the solubility ratio—expressed as mole fraction in water, N_0, and in ethanol, N_A—of glycine may be compared

[1] There is no adequate translation of the word "Zwitterion". We shall tentatively adopt the term "dipolar ion" introduced by Ingold, although it is not an ideal description of this class of molecules.

with glycolamide, of alanine with lactamide, and of norleucine with α-hydroxycaproamide (table 1). These amides of α-hydroxy acids have the same composition as, though different structures than, the α-amino acids. The comparison of solubilities in water and ethanol is somewhat unsatisfactory, however, because certain of the molecules are very soluble in water (43). Under these circumstances, solubility ratios may not yield activity

TABLE 1

Influence of dipolar ionization estimated by comparison of α-amino acids and α-hydroxy amides

α-AMINO ACIDS	LOG N_A/N_0	AMIDES OF α-HYDROXY ACIDS	LOG N_A/N_0	INFLUENCE OF DIPOLAR IONIZATION Δ LOG N_A/N_0
Glycine 	−3.391	*Glycolamide* 	−0.799	−2.592
Alanine 	−2.856	*Lactamide* 	−0.254	−2.602
Norleucine 	−1.414	*α-Hydroxycaproamide* 	+1.084	−2.498

ratios. None the less, the solubility ratio N_A/N_0 is consistently from three hundred to four hundred times greater for the α-hydroxyamides than for the α-amino acids.

The comparison of amino acids and peptides with their hydantoic acids is more satisfactory, for, although the hydantoic acids prepared from them differ not only by the shift in proton characteristic of dipolar ion structure

TABLE 2

*Influence of dipolar ionization estimated by comparison of amino acids and peptides with hydantoic acids**

SUBSTANCE	LOG OF SOLUBILITY RATIO LOG N/N_0	INFLUENCE OF DIPOLAR IONIZATION
Formamide		
Glycine	−1.229	
Hydantoic acid	+0.755	−1.98
α-Aminocaproic acid	−0.360	
α-Aminocaproic hydantoic acid	+1.728	−2.09
Methanol		
Glycine	−2.515	
Hydantoic acid	−0.271	−2.24
α-Aminocaproic acid	−0.657	
α-Aminocaproic hydantoic acid	+1.568	−2.23
Ethanol		
Glycine	−3.391	
Hydantoic acid	−0.630	−2.76
α-Alanine	−2.856	
α-Alaninehydantoic acid	−0.139	−2.72
β-Alanine	−3.139	
β-Alaninehydantoic acid	−0.463	−2.68
α-Aminocaproic acid	−1.414	
α-Aminocaproic hydantoic acid	+1.352	−2.77
Diglycine	−4.367	
Diglycinehydantoic acid	−1.533	−2.83
Triglycine	−4.965	
Triglycinehydantoic acid	−2.253	−2.71
Butanol		
Glycine	−3.808	
Hydantoic acid	−1.011	−2.80
α-Aminocaproic acid	−1.711	
α-Aminocaproic hydantoic acid	+1.118	−2.83
Acetone		
Glycine	−4.401	
Hydantoic acid	−1.520	−2.88
α-Aminocaproic acid	−2.432	
α-Aminocaproic hydantoic acid	+0.437	−2.87

* These solubility data are reported in more detail in references 15, 41, and 42.

but also by a CONH group, this represents a constant difference estimated at +0.14 from comparison of glycolamide or lactamide with hydantoic acid and methylhydantoic acid. Diglycine is isomeric with methylhydantoic acid, that is, the hydantoic acid derived from α-alanine, as well as that from β-alanine. Their comparison is, however, not satisfactory, since both the CONH and the CH_2 groups require different amounts of work for their transfer, depending upon their position in the molecule (42). Comparison of a large series of amino acids and peptides with the hydantoic acids prepared from them (by treatment with potassium cyanate under the appropriate conditions) yields the consistent difference for the transfer from water to various other solvents shown in table 2. These studies suggest that, within the limits of error of the measurements, that part of the free energy of transfer from water to another solvent due to electrostatic forces is independent of the dipole moments of these molecules and of the

TABLE 3

Influence of CH_2 groups and of dipolar ion structure on change of free energy with change in solvent

SOLVENT	DIELECTRIC CONSTANT	INFLUENCE OF CH_2 GROUPS		INFLUENCE OF DIPOLAR IONIZATION	
		$\log \frac{\Delta N}{N_0}$	ΔF calc.	$\log \frac{\Delta N}{N_0}$	ΔF calc.
Formamide	>84	+0.23	314	−2.03	−2768
Methanol	32.71	+0.44	600	−2.24	−3054
Ethanol	24.28	+0.49	668	−2.73	−3722
Acetone	20.83	+0.49	668	−2.87	−3913
Butanol	17.51	+0.53	723	−2.82	−3845
Heptanol	9.33	+0.53	723		

number of CH_2 groups in the paraffin chain. The result is the same if we compare glycine or norleucine, diglycine or triglycine, with their hydantoic acids. And the result is of the same order of magnitude whether the comparison is with hydantoic acids or α-hydroxyamides.

The same measurements may be employed in calculating the work involved in the transfer for each additional CH_2 group in paraffin chains ending in methyl groups.[2] This change in free energy has the opposite sign from that due to dipolar ionization and is the same whether the CH_2 groups are on uncharged molecules or dipolar ions.

The influences of CH_2 groups, and of dipolar ionization, upon the free energy of transfer to a variety of solvents estimated by comparison of α-amino acids and their derivatives are summarized in table 3.

[2] For CH_2 or CONH groups situated between polar groups the problem is far more complicated (42) and is not considered here.

These results are far simpler than could have been predicted on the basis of theoretical considerations. They do not reveal the expected influence of the dipole moments of the molecules on the free energy of transfer (34, 54), nor, in the case of the more soluble molecules, the influence of dipolar ions on each other in their saturated aqueous solutions. The latter effect has been estimated to be 0.15 for glycine in water from freezing-point measurements (55), and to be 0.13 from vapor-pressure measurements at 25°C. (57). Moreover, change in $\log N/N_0$—or in $\Delta\bar{F}$—is not proportional to the reciprocal of the dielectric constant. Thus the characterization of the solvent in terms of its dielectric constant is a less satisfactory approximation than in the case of ions.

When one considers that the electrostatic forces surrounding dipolar ions are of shorter range than those surrounding ions (34, 54), whereas their dimensions are far greater (11, 13), it is obvious that Coulomb forces cannot be expected as nearly to explain the behavior of dipolar ions as of ions. Among dipolar ions, those with electric moments large in comparison with their volumes behave most like ions, whereas those with moments small in comparison with the length of their paraffin side chains behave most like uncharged organic molecules.

Solubility in ethanol–water mixtures

Solubility in three-component systems is more complicated than in the two-component systems thus far considered. Besides the forces between solute molecules and the two solvent species, there are the forces between the solvent molecules themselves. Moreover, if the affinity of the solute is far greater for the one than for the other solvent, a redistribution of solvent molecules may occur in the neighborhood of solute molecules so that the solvent as a whole can no longer be considered a uniform medium.

None the less, studies upon amino acids, peptides, and their derivatives in ethanol–water mixtures illustrate many of the types of behavior with which we are concerned, and enable us to distinguish, at least qualitatively, between the forces due to the charged groups of dipolar ions and those due to the paraffin chain. When the logarithm of the solubility ratio of glycine and its peptides is plotted as ordinate against the mole fraction of ethanol in the solvent (figure 1) the three curves are very similar. All have steep segments at low mole fractions of alcohol, with comparable points of inflection in the range in which the solvent molecules are approximately equal in number. In systems containing larger amounts of ethanol the logarithm of the solubility may, as a first approximation, be considered to vary inversely as the mole fraction of ethanol. Straight lines have been drawn through these segments of the curves of glycine and its peptides.

The curves describing the behavior of formyl derivatives of the amino acids, their hydantoins and hydantoic acids in ethanol–water mixtures

(41, 42) also form a family, though their shape is quite different from that of the amino acids and peptides from which they were derived. For, whereas small amounts of alcohol diminish the solubility of α-amino acids and the peptides of glycine, they increase the solubility of their derivatives

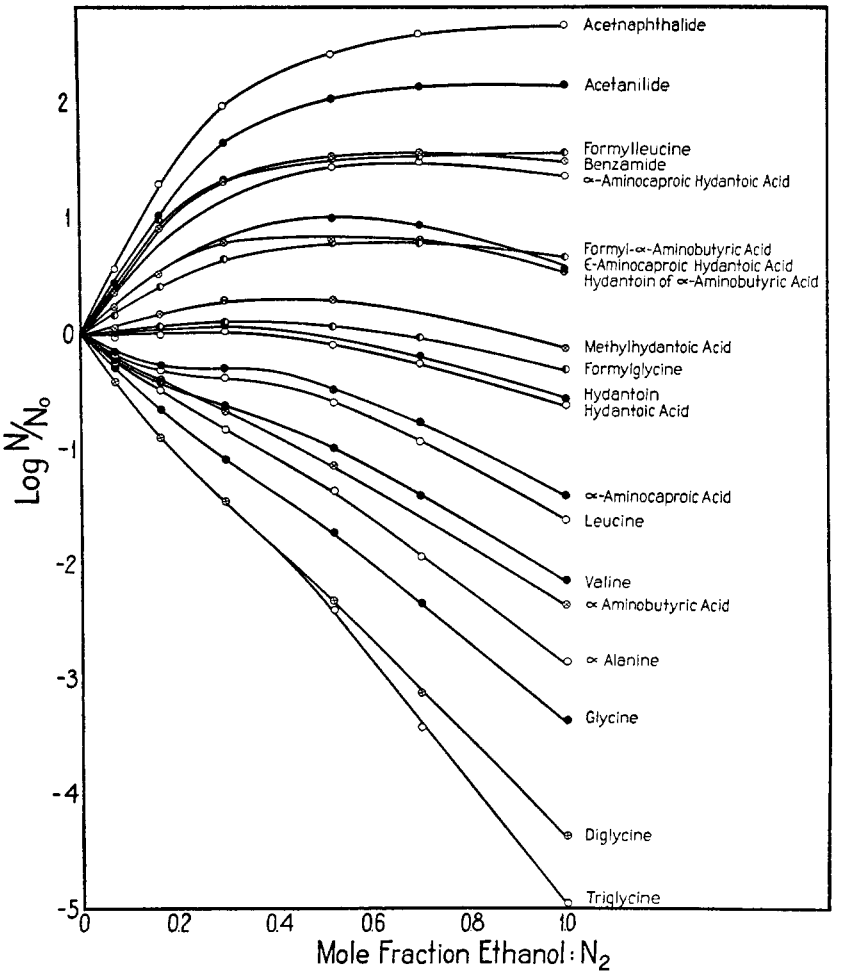


FIG. 1. Solubility of amino acids, peptides, and related substances in ethanol-water mixtures at 25°C.

which are no longer dipolar ions. This is particularly marked with α-aminocaproic hydantoic acid. The additional CH_2 groups of this molecule as compared with hydantoic acid are reflected by increased solubility in systems rich in ethanol. The curve for this substance is very similar to

that for formylleucine or benzamide (41) and such other typical organic compounds as acetanilide and acetnaphthalide. The isomer, ϵ-aminocaproic hydantoic acid, in which the CH_2 groups lie between polar groups, behaves far more like the formyl derivative or hydantoin of α-aminobutyric acid (42); that is to say, like a molecule with two less CH_2 groups. Its solubility in 80 per cent ethanol is approximately tenfold that in water and threefold that in ethanol, a type of behavior characteristic of a class of proteins, the prolamines.

All naturally occurring α-amino acids are less soluble in ethanol–water mixtures than in water.[3] Whereas small amounts of ethanol greatly diminish their solubility, larger amounts have less effect the longer the paraffin side chains of the molecule (figure 1). Dipolar ions differing from each other only by the number of CH_2 groups in paraffin chains ending in methyl groups, n_{CH_2}, are related in their solubility behavior to glycine by the following approximate rule:

$$\log (N/N_0)_{\text{glycine}} = \log (N/N_0)_{\alpha\text{-amino acid}} - 0.49\, v_2^2\, n_{CH_2} \qquad (3)$$

where all solubilities are given in mole fractions and v_2 is the volume fraction of ethanol in the solvent (41). In absolute alcohol the above equation reduces to the rule, and yields the value given in table 3. Whether the same rule with the coefficient characteristic of the other pure solvents studied will hold also for their mixtures with water remains to be investigated.[4] It is also possible that comparable relations may obtain between various tripeptides and triglycine. In any case it would appear possible to analyze solubility ratios of α-amino acids in various solvents in terms of the charged groups and the length of the paraffin side chains in which they differ from each other. In these terms, also, one may hope to be able to analyze the behavior of still more complicated peptides, phospholipoids, and proteins.

II. CHANGE IN FREE ENERGY WITH CHANGE IN IONIC STRENGTH AND DIELECTRIC CONSTANT

The influence of neutral salts in increasing the solubility of proteins is so marked that it was observed in the middle of the last century. In 1859

[3] The rules that have been deduced (table 3) demand that this should not be true for α-amino acids containing more than eight CH_2 groups, and measurements upon α-aminostearic acid demonstrate that it is far more soluble in ethanol than in water.

[4] The logarithm of the ratio of solubility of α-amino acids in butanol saturated with water, to that in water (23), has a coefficient close to 0.4 for the CH_2 group, whereas the coefficient for formyl amino acids in pure butanol is 0.53. The volume fraction of butanol in a solution saturated with water is 0.835. Neglecting the effect of the amino acid on the solubility of water in butanol, and assuming the same equation to hold for butanol–water as for ethanol–water mixtures, we should have:

$$0.53\, v_2^2\, n_{CH_2} = 0.53 \times 0.835^2 n_{CH_2} = 0.37\, n_{CH_2}$$

Denis, a French scientist, noted that certain of the proteins of the blood were insoluble in water, but soluble in dilute salt solutions. Proteins that behave in this way are classified as globulins. As a result of a study upon serum globulin in 1905 Mellanby concluded: "Solution of globulin by a neutral salt is due to forces exerted by its free ions. Ions with equal valencies, whether positive or negative, are equally efficient, and the efficiencies of ions of different valencies are directly proportional to the squares of their valencies" (44, p. 373). This accurate formulation of the principle of the ionic strength—rediscovered in 1921 by G. N. Lewis (38) as a description of the effects of neutral salts upon each other—acquired theoretical significance in Debye's theory of interionic forces. The ionic concentration, Γ, defined as the summation ΣCz^2, where z is the valence of each ionic species, is related in Debye's theory to the temperature, the dielectric constant of the medium and κ, a measure of the thickness of the ion atmosphere, by the equation:

$$\kappa^2 = \frac{4\pi N\epsilon^2}{1000\,DKT} \sum Cz^2 = \frac{12.67 \times 10^{18}}{DT} \tag{4}$$

The dielectric constant thus plays an important rôle in this theory in determining that part of the activity coefficients of ions due to Coulomb forces, fe.

$$-\log fe = \left(\frac{\epsilon^2 z^2}{2.303 \times 2DKT}\right)\left(\frac{\kappa}{1 + \kappa a}\right) \tag{5}$$

In this equation the dielectric constant is considered uneffected by change in ionic strength. If the dielectric constant of the medium also changes, a constant comparable to that employed by Debye and McAulay (22) and by Hückel (31) must also be added.

Since biological systems contain ions of various kinds as well as other components, some of which presumably decrease and others of which are known to increase the dielectric constant of water, the significance of these relations can scarcely be overemphasized. Were the interaction between dipolar ions and ions completely defined by the above equation, and were it necessary merely to substitute in equation 5 the change in the dielectric constants of solutions due to biological components in order to estimate the change in the activity coefficients of ions in biochemical systems, the problem would indeed be simple, at least in sufficiently dilute salt solutions. The influence of different ions would then depend upon their radii, b, their mean effective diameters, a, and valence, z, and the dielectric constant.

The Coulomb forces due to ions are not, however, the only ones which we must consider. The conditions in which the activity coefficients of ions and dipolar ions may be expected to depend most completely upon Coulomb forces will, however, be considered first. These obtain in media of

low dielectric constant at low temperatures. Conversely, the specific properties of ions and dipolar ions manifest themselves the more the higher the temperature and the dielectric constant.

In dilute solutions of electrolytes the logarithm of the activity coefficient is proportional to κ and to the square root of the ionic strength. In the case of dipolar ions the logarithm of the activity coefficient does not vary as the square root, but as the first power of the concentration. This was empirically discovered by studying the solvent action of neutral salts upon cystine, and theoretically demonstrated by Scatchard and Kirkwood (54) in an extension of the theory of Debye and Hückel to dipolar ions. They demonstrated that the term proportional to the square root of the concentration vanishes when the net charge is zero.

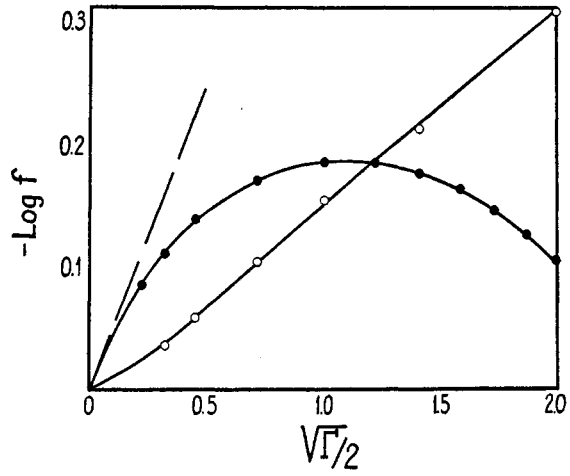


FIG. 2. Activity coefficients: ●, of sodium chloride (Harned and Nims: J. Am. Chem. Soc. **54**, 423 (1932)); ○, of cystine in sodium chloride.

The activity coefficients of cystine in the presence of sodium chloride, estimated by the solubility method, are compared with those of sodium chloride in the accompanying figure, so plotted as to demonstrate how completely this dipolar ion deviates from the square root law applicable to ions (figure 2). The activity coefficients of most amino acids cannot be determined in water by the solubility method because of their high solubility. The activity coefficients of glycine in sodium chloride solution have, however, been calculated by Scatchard and Prentiss (55) from freezing-point measurements and by Joseph (33) from electromotive force measurements at 1.4°C. in cells with amalgam electrodes, but without liquid junctions. The results of these two investigations are completely in accord and yield precise information regarding the influence of salt upon glycine and of glycine upon salt in aqueous solution.

Influence of sodium chloride upon glycine in media of varying dielectric constant

Most amino acids, peptides, and proteins are sufficiently insoluble in ethanol–water mixtures not to contribute appreciably to the dielectric constant of such solutions. Under these conditions the properties of the solutions approach those of the pure solvents. Glycine is soluble in water to the extent of 2.886 moles per liter. In 60 per cent ethanol its solubility is 0.157, in 80 per cent 0.0278, and in 90 per cent 0.0056 moles per liter (15).

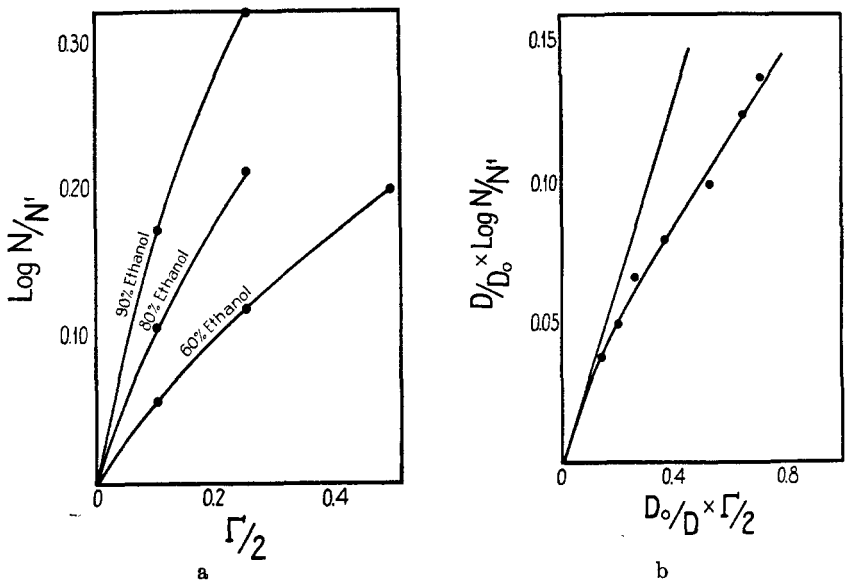


FIG. 3. Interaction of glycine in lithium chloride and ethanol–water mixtures: 3a, uncorrected; 3b, corrected for the influence on activity coefficients of the dielectric constant.

The solvent action of neutral salts upon glycine is greater the lower the dielectric constant of the medium. This is demonstrated if the ratio of the solubility in the ethanol–water mixture containing neutral salt, N, to that in the same ethanol–water mixture, N', is plotted against the concentration of salt (figure 3a).

If the logarithm of the solubility ratio is multiplied by the dielectric constant ratio $[(D/D_0) \log N/N']$, and plotted against $(D_0/D)\ \Gamma/2$ the curves for glycine in 60, 80, and 90 per cent ethanol containing lithium chloride coincide within the limit of error of the measurements (figure 3b). We may, therefore, conclude that beyond 60 per cent ethanol changes in the activity coefficient of glycine with change in ionic strength are largely ascribable to Coulomb forces.

The addition of salt to an alcohol–water mixture increases the volume. Some decision must therefore be made as to the manner in which to compute the dielectric constant in the four-component systems here considered. Since dielectric constant measurements cannot be made in the presence of electrolytes, it remains an open question whether solutions would be more nearly isodielectric where the volume occupied by the salt is neglected, as well as any contribution of the salt to the dielectric constant, or whether the volume occupied by the salt should be considered to have the same properties as water and the sum of water plus salt be retained constant. Measurements have been made in systems constituted in both ways, and the solubility, computed in solvents defined in either way, yields the same results. All of the curves extrapolate to closely the same value for the limiting slope, which may be taken as 0.30 ± 0.02 regardless of the ethanol–water mixture, or of the method of defining the systems.

Influence of different alkali halides upon glycine

Kirkwood has developed an equation for the interaction between ions and dipolar ions and applied it to precisely these measurements upon glycine (34). In his equation the first term for the logarithm of the activity coefficient of a spherical dipolar ion increases with the square of its dipole distance R, divided by a, the sum of the radii of ion and dipolar ion. The greater the dipole moment the greater the solvent action of neutral salts. Moreover, if the center of the dipole be considered the center of the molecule, as in glycine and presumably many spherical proteins, this effect at low ionic strengths will dominate all others, since R^2 will be large in comparison with a.

The change in free energy is a very complicated function of the ionic strength in the Kirkwood equation, and this is especially true if the charged groups of dipolar ions are at the edge of the molecule. Under these circumstances the multipole moments cannot be neglected. If the limiting slope alone is considered, all terms up to the octopole moments being included, Kirkwood's expression may be written:

$$\frac{(D/D_0)\log fe}{(D_0/D)\,\Gamma/2} = -0.125\,\frac{R^2}{a}\left\{1 + \frac{20}{27}\left(\frac{\rho}{a}\right)^2\left[1 - \frac{R^2}{4\rho^2}\right] + \frac{7}{10}\left(\frac{\rho}{a}\right)^4\left[1 - \frac{5R^2}{8\rho^2} + \frac{5R^4}{48\rho^4}\right]\right\} \tag{6}$$

where ρ is the distance of the charged groups from the center of the molecule. The distance from the edge of the molecule, a value that is probably fairly constant for amino acids, peptides, and proteins, is therefore $(b - \rho)$.

Kirkwood's analysis of our curve for glycine yielded 10 A.U.[2] as an esti-

mate for R^2 or 3.17 A.U. for R. This is equivalent to a moment of 15×10^{-18} E.S.U. We have now studied the influence of other alkali halides on glycine, with the intention of varying this parameter in the above equation. Taking the radius of glycine to be 2.82 A.U. (13) and the radii of the salts, b, to be those estimated by Pauling (50), one obtains the estimates of $0.125/a$ shown in table 4. Assuming R^2 to be 10 A.U., the limiting slope of glycine in lithium chloride would be 0.32, in good agreement with our experimental findings. The studies upon sodium chloride and potassium chloride do not reveal lower but rather higher limiting slopes (0.31 ± 0.01) than for lithium chloride,[5] and curvature is very similar, although, according to the theory, deviation from the limiting slope should be greater the larger the value of a.

TABLE 4

Sum of the radii of glycine and alkali halides

HALIDES	RADII OF IONS b	SUM OF RADII OF GLYCINE AND IONS $(2.82 + b)$ a	$0.125/a$
	A.U.	*A.U.*	
LiCl	1.082	3.90	0.0321
NaCl	1.231	4.05	0.0309
KCl	1.381	4.20	0.0298
LiBr	1.138	3.96	0.0316
NaBr	1.288	4.11	0.0304
KBr	1.438	4.26	0.0293
LiI	1.221	4.04	0.0309
NaI	1.370	4.19	0.0298
KI	1.520	4.34	0.0288

The radius of lithium chloride Pauling estimates to be the smallest among the alkali halides, and that of potassium iodide to be more than a third again as great, namely, 1.52 A.U. According to equation 6 the limiting slope should therefore be approximately 10 per cent smaller and the curvature should also reflect the larger value of a. The measurements upon the interaction of potassium iodide and glycine in ethanol (figure 4) reveal an appreciably lower limiting slope than in the case of sodium chloride or potassium chloride. The interaction of other alkali halides, as

[5] In water, where these salts differ from each other far more in their influence upon amino acids and proteins, the solvent action of lithium chloride is greater than that of sodium chloride and that of sodium chloride than that of potassium chloride, and their salting-out effect is in the reverse order.

well as salts of other valence types with glycine and also with other amino acids and peptides, is being further investigated. Studies upon the influence of calcium chloride upon glycine in 80 per cent ethanol suggest that the solvent action of bi-univalent salts is greater than that of uni-univalent salts in this solvent as in water.

Influence of salt upon cystine

Most naturally occurring amino acids are less soluble in water than glycine; the imino acids proline and hydroxyproline are exceptions, as well as lysine and arginine, which are not α-amino acids. Solubility of amino acids in water is, as we have seen, generally decreased with increase

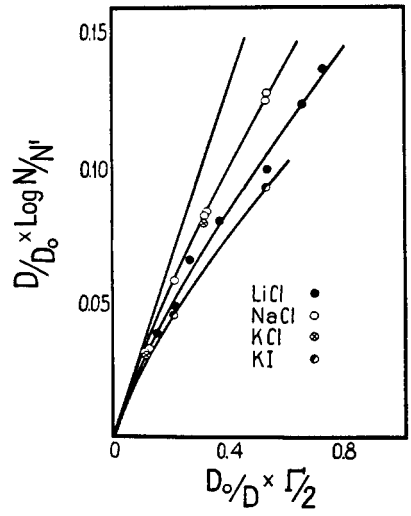


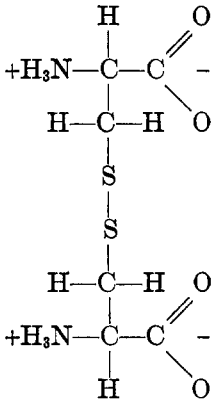
FIG. 4. Interaction of glycine and alkali halides in ethanol–water mixtures

in the number of CH_2 groups in the molecule. The longer the paraffin chain the greater the salting-out effect of neutral salts upon amino acids and this is greatest, as in the case of the proteins, for uni-bivalent salts, such as phosphates and sulfates and least, as has long since been shown by Pfeiffer and his collaborators (51), for bi-univalent salts, such as calcium chloride or strontium nitrate.

In order to determine the conditions under which the principle of the ionic strength—first described in connection with the solubility studies upon the proteins—obtains for amino acids, it seemed desirable to study a molecule which was relatively insoluble in water, but which possessed no long paraffin chains. For, the higher the solubility in water and therefore the dielectric constant of the medium, the smaller the Coulomb forces.

Conversely, the longer the paraffin chains of dipolar ions, the lower the dielectric constant of the medium must be for Coulomb forces to dominate the salting-out effect.

Cystine is the least soluble of the naturally occurring amino acids, having a solubility in water of 0.109 g. per liter at 25°C. It is a tetrapole, consisting essentially of two α-alanine molecules coupled by an S—S linkage.



Its electric properties might be expected to be determined by the configuration of the four charges constituting the two dipole pairs. Each of these may be expected to have moments comparable to that of glycine, but the vector sum of these moments might be zero or double that of glycine. The molal volume of cystine may be estimated to be 156 cc.[6] and, considered as a sphere, its radius would therefore be 3.94 A.U.

The influence of calcium chloride upon the solubility of cystine was studied some years since by Blix (5). McMeekin has confirmed his measurements and studied the influence of a large number of other salts on the solubility of cystine in water (figure 5). The fan-like spread of solubility in the presence of different salts at the same ionic strength—multivalent cations having the greatest, and multivalent anions the smallest solvent action—resembles that characteristic of certain proteins and is considered in a later section of this paper.

The principle of the ionic strength can scarcely be said to be illustrated by salts in this medium at the concentrations studied. At the lowest calcium chloride concentrations studied by Blix, 0.063 mole per liter, the solubility is appreciably higher than at the same ionic strength of sodium chloride. The curvature in the case of the sodium chloride was, however,

[6] Taking the S–S volume as 29.8 cc., as in α,α'-dithiodiacetic acid (30) and the volumes of the other groups as previously given (13).

far greater, and there was no evidence therefore that the limiting slopes would be different.

In order to determine the limiting slope more accurately the solvent action of sodium chloride on cystine was studied in 30 per cent ethanol. The results, very close to those of calcium chloride in water,[7] suggest a limiting slope of 0.42, or something less than half again that of glycine. Assuming cystine to be a sphere, the sum of its radius, 3.94 A.U., and that of sodium chloride, 1.23 A.U. (50), yields a value of 5.17 A.U. for a. Taking 0.42 as the limiting slope for cystine in sodium chloride and putting it equal to R^2/a in Kirkwood's equation, R^2 is equal to 17.37 A.U. and R to 4.17 A.U. This calculation, though too simple, suggests that the two dipoles in cystine are neither parallel nor anti-parallel. They presumably

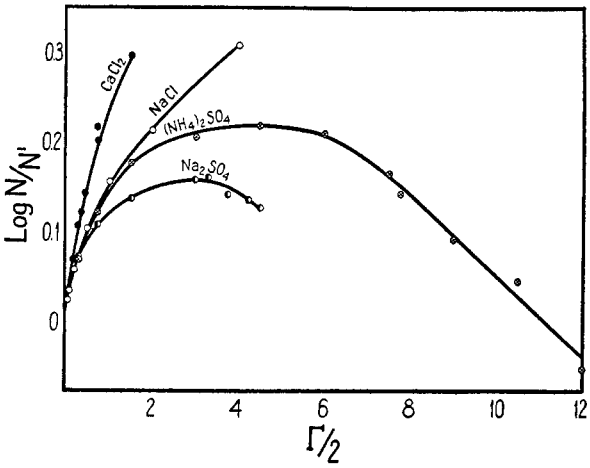


FIG. 5. Solubility of cystine in aqueous salt solutions

are at an angle with respect to each other as a result of rotation around the S—S bond. In order to study more exactly the influence of dipole moment upon the interaction with salts we therefore turned our attention to molecules in which the amino and carboxyl groups are separated by hydrocarbon and peptide chains.

Influence of salt upon peptides of varying dipole moment

The dipole moments of peptides increase with the number of amino acid residues bound in the chain. In the stretched condition the distance

[7] Incomplete measurements upon cystine in 30 per cent ethanol containing calcium chloride suggest a slightly higher limiting slope of 0.48. If the salting-out constant K_S for cystine in sodium chloride, 0.14, be added to the apparent value, KR', of 0.42, a still higher value of 0.56 is obtained for the true limiting slope, KR, and an estimate of R of 4.81 A.U. (See equation 8.)

separating the positive and the negative charge of these dipolar ions will therefore be greater for diglycine than for glycine, and for triglycine than for diglycine. Such molecules can, however, by no means be considered spherical. Rather they resemble cylinders of constant radius (13) and of the lengths estimated in table 5.

The peptides of glycine and the tetrapole lysylglutamic acid, which contains two negatively charged carboxyl and two positively charged

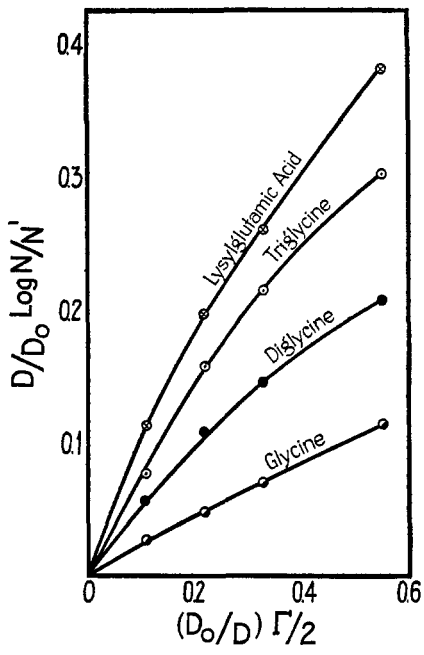


FIG. 6a

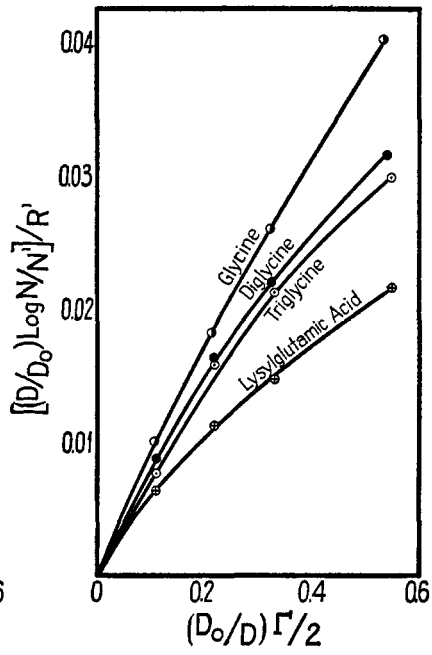


FIG. 6b

FIG. 6a. Solubility ratios, in 80 per cent ethanol containing sodium chloride, of amino acids and peptides.

FIG. 6b. Solubility ratios, in 80 per cent ethanol containing sodium chloride, of amino acids and peptides divided by their dipole distances as estimated by structural considerations.

ammonium ions and has still larger electric moments (30), have been studied in 80 per cent ethanol containing sodium chloride at 25°C. The results are graphically represented in figure 6a, those for glycine being added for comparison. These measurements leave no doubt that the interaction of ions and dipolar ions is accompanied by greater change in free energy the greater the moments of the dipolar ions.

Although it is certain that the longer peptides have longer dipole moments, no satisfactory method is available for estimating the moments of

dipolar ions. In a recent discussion of the problem two approximations were compared (11). In the one the molecules are considered extended in solution, and the increment in the distance between the charged groups taken as 1.26 A.U. for each CH_2 group, and as 2.34 A.U. for each CONH group. Taking the dipole distance R of glycine as 3.17 A.U., that of diglycine is 6.67 and of triglycine 10.17. On this basis the longest dipole of lysylglutamic acid is 14.23 A.U., and the shorter one, which has the same configuration as diglycine, has a length in the direction of the chain of 3.5 A.U. The vector sum of these is therefore 17.73 A.U. (30).

The ratio $[(D/D_0) \log N/N']/R'$, where R' represents the estimated dipole distance in the stretched condition, is plotted in figure 6b against (D_0/D) $\Gamma/2$. The agreement at low concentrations of salt indicates that *the logarithms of the activity coefficients of amino acids and peptides are, as a first approximation, proportional to their dipole moments.* Moreover, these estimates of dipole distance may be considered maximal. Presumably there is at least some bending around the free bonds in these molecules, resulting in smaller dipole moments than these estimates, especially for the longer molecules.[8] Eyring (25) and Kuhn (37) have estimated the amount of such bending from statistical considerations. Correcting for this effect would bring the peptide solubility curves, plotted as in figure 6b, still closer together and render the shapes of the molecules in solution rather elliptical than cylindrical.

Kirkwood has recently extended his treatment to an elliptical model and concluded that for long molecules the logarithm of the activity coefficient is proportional at low ionic strengths to R. Theory and experiment are thus in agreement that, for such molecules as the peptides that have thus far been studied, change in free energy with change in ionic strength due to Coulomb forces is proportional to the dipole distance R, whereas for spherical dipolar ions the limiting slope for a single dipole is proportional, according to Kirkwood's theory (34), to R^2/a.

Influence of salt upon proteins

Proteins, like the amino acids of which they are composed, vary with respect to their solubility in water, in alcohol–water mixtures, and in salt solutions. There is no reason to believe that the principles that apply in the case of amino acids do not hold also in the case of proteins, though the complexity of the analysis is of necessity far greater because of the vast size of the protein molecule, and the larger number of positively and negatively charged groups on its surface, even in the isoelectric condition. Whereas the radii of simple ions, considered as spheres, vary from 1.0 to 2.5 A.U.

[8] The approach to this problem through the dielectric constant increments, δ, of dipolar ions is considered elsewhere in this paper.

and those of amino acids from 2.8 to approximately 4.0 A.U., the radius of egg albumin is estimated by Svedberg to be 22 A.U. (60), of hemoglobin 27 A.U., of edestin 39.5 A.U., and of the hemocyanin of Helix 120 A.U (58). Certain proteins, among them the fibrinogen of blood (7), which is concerned with its coagulation, and the globulin of muscle, which is concerned with its contraction (46), far from being spherical, are rod-shaped, giving rise to double refraction of flow, the latter having a length that has been estimated[9] at 6000 A.U.

A spherical protein is now best considered as a coiled polypeptide chain, held in its native form either by hydrogen bonds (45), by other forces between juxtaposed peptide linkages, or by electrostatic forces between its charged groups[10] (14). Since the volumes of spheres increase with the third power of the radius, the effect of each protein molecule in displacing solvent is very great. The specific volumes of most proteins are close to 0.75.

The number of electric charges on most simple ions is one, two, three, or at most four or five. Egg albumin, a quite small protein, which is very soluble in water, but readily crystallizable from concentrated salt solutions, has approximately twenty-seven dissociable basic and twenty-seven dissociable carboxylic groups. Although the radius of egg albumin is approximately eight times that of the smallest amino acid, glycine, it has at least twenty-seven times as many charged groups.

It is not, however, the number of charged groups, but their distribution on the surface of molecules and the resultant electric moments that determine the solubility of amino acids, and presumably of proteins. The greater the electric moments of amino acids of equal crystal lattice energies, and the smaller the volume of non-polar groups in the molecule, the greater the solubility in water.

Hemoglobin, the iron-containing oxygen-combining protein of the blood, possesses a larger number of dissociable groups. It has approximately eighty-seven dissociable acid and an equal number of dissociable basic groups, but not more than seventy-five of these appear to form dipole pairs at the isoelectric point (12). Although the molecular weight of horse hemoglobin has been reported to be approximately equal, under certain conditions (9), to that of myoglobin and of egg albumin (58, 59), both

[9] This estimate has been made by Edsall on the basis of measurements of double refraction of flow (46) and Werner Kuhn's theory (36).

[10] From this point of view distortion of the native form occurs when protein is spread on a surface layer, and reveals the dimensions of the polypeptide chain (28, 32, 47). Increase in the heat motion in the case of these molecules or decrease in the dielectric constant of the medium leads also to denaturation, the resulting molecular configurations having far lower solubilities and presumably far smaller electric moments.

ultracentrifugal and osmotic-pressure measurements suggest that the normal molecular weight is 67,000 (1, 61). The molecular volume would therefore be 50,000 and the radius, calculated as a sphere, 27 A.U. Hemoglobin is, however, not as symmetrical a molecule as egg albumin (58).

The hemoglobin of the horse is soluble in water at 25°C. approximately to the extent of 17 g. per liter. In the presence of 1.05 moles of sodium chloride it is soluble to the extent of 198 g. per liter (29). Solubility is thus increased approximately tenfold by 1 mole of salt or to the extent characteristic of the influence "of a neutral salt upon a bi-bivalent or uni-quadrivalent compound" (17). The solubility of hemoglobin is thus so greatly increased by neutral salts as to warrant its characterization as a globulin. Its solubility in water is not sufficiently low, however, to demonstrate

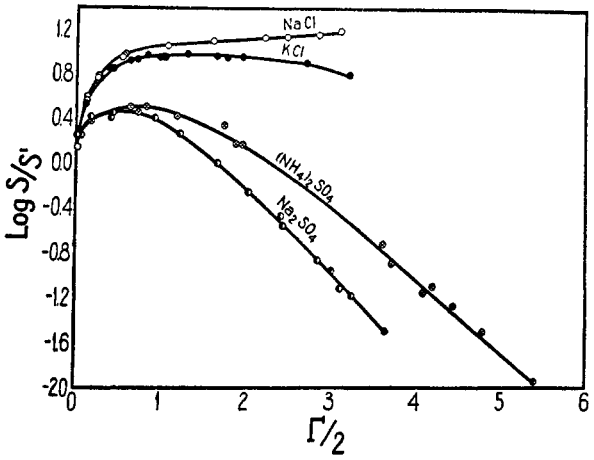


FIG. 7. Solubility of carboxyhemoglobin in aqueous salt solution

unequivocally the principle of the ionic strength under these conditions. For hemoglobin as for cystine the different solvent action of different salts is clearly demonstrated by the extensive investigations that have been carried on in aqueous solutions. The comparable nature of the salt effect upon amino acids and proteins is illustrated by figures 5 and 8. The curves in both cases have comparable contours, chlorides having the greatest solvent action of the salts studied, and the solvent action of the sulfates being overshadowed by their salting-out effect. In the case of hemoglobin, not only is the solvent action greater than in the case of cystine, but the salting-out effect becomes manifest at even lower concentrations. The serum globulin studied by Mellanby (44), which led him to formulate the principle of the ionic strength, had a far smaller solubility than hemoglobin, and the solvent action of neutral salts upon it was far greater.

Hemoglobin is sufficiently soluble to increase appreciably the dielectric constant of its saturated aqueous solutions. It therefore seemed desirable to determine its interaction with sodium chloride in a solvent of lower dielectric constant. The solubility of hemoglobin is estimated to be 0.036 g. per liter in 25 per cent ethanol at −5°C. (27). The logarithm of the ratio of its solubility in solutions containing salt to that in the salt-free ethanol–water mixture, multiplied by the dielectric constant ratio, is plotted in figure 8 against the sodium chloride concentration also multiplied by the dielectric constant ratio. Comparison with figure 7 demonstrates that the solvent action of sodium chloride is not only greater at the lower

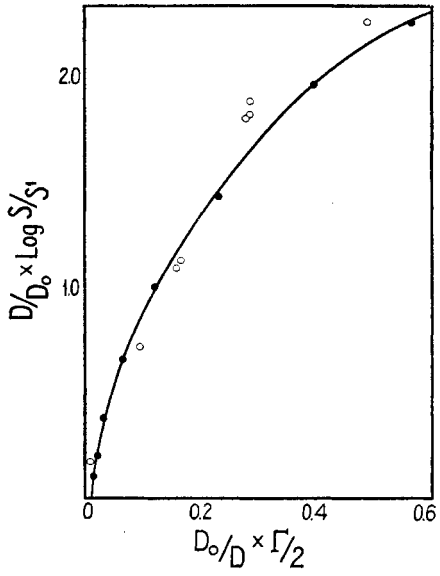


FIG. 8. Solubility ratio of carboxyhemoglobin in 25 per cent ethanol (●) compared with measurements (○) in aqueous solution (29) corrected by assuming 30,000 as the value of δ (48).

dielectric constant, but that it is greater even when the dielectric constant is corrected for, in the above manner. In order to account for the discrepancy one may assume that the solutions containing hemoglobin in water have a dielectric constant greater than that of water by approximately 30,000 per mole of hemoglobin.

The solubility measurements of Green (29) upon carboxyhemoglobin in aqueous sodium chloride, corrected on the assumption that the dielectric constant increment, δ, of this protein is 30,000 (figure 8) fall satisfactorily on the curve drawn through the points in 25 per cent ethanol, where the hemoglobin is too insoluble to contribute appreciably to the dielectric

constant of the solution. Nor is such a high value for δ fantastic. The dispersion curve upon this protein of Errera (6, 24), confirmed in our laboratory by Oncley (48), extrapolates to the slightly higher value of 35,000 $\pm$ 5000. The higher value of δ yields still greater estimates for $(D/D_0) \log S/S'$ in water. The dielectric constant of the most concentrated hemoglobin solution considered is on this basis 182 as compared with 87 in the absence of salt, and there is no present method of correcting the value of S' in water to the same dielectric constant as S. Moreover, a salting-out effect due to interaction with sodium chloride in solutions of high dielectric constant must, of course, also be assumed. These ten-

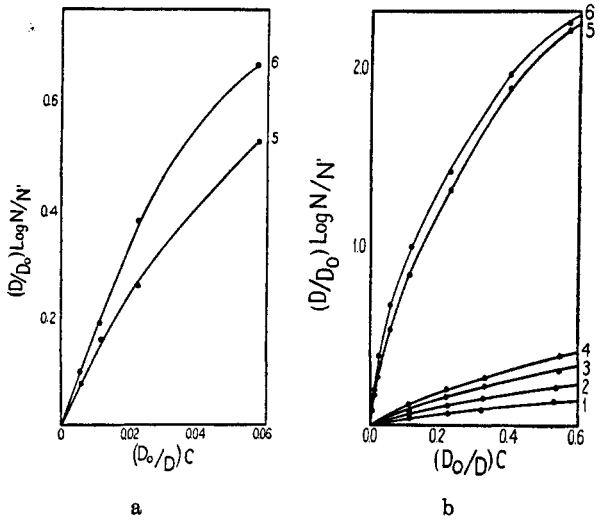


FIG. 9. Activity coefficients of: 1, glycine; 2, diglycine; 3, triglycine; 4, lysylglutamic acid; 5, egg albumin; and 6, carboxyhemoglobin in ethanol–water mixtures containing sodium chloride. (a) Proteins in dilute salt solutions. (b) More concentrated salt solutions, in which amino acids, peptides, and proteins have been studied.

tative calculations are presented as indicating the importance of the dielectric constant in influencing interaction with salts in concentrated protein solutions and presumably in biological systems.

Albumins are by definition water-soluble proteins. Forty per cent solutions of salt-free isoelectric egg albumin are readily prepared. Like other proteins, however, the solubility of egg albumin is greatly reduced by even small concentrations of ethanol. In 25 per cent ethanol at -5°C. (the same conditions that obtained in the hemoglobin study) the solubility of egg albumin was only 0.13 g. per liter. Under these conditions neutral salts increased its solubility many fold, as they do with globulins in aqueous solution.

The results upon egg albumin are also consistent with the prediction that in the interaction between ions and dipolar ions the logarithm of the solubility ratio is proportional to the ionic strength and not to its square root (27). At high salt concentrations, where Debye's law for ions would not be expected to hold, the logarithm of the solubility does, however, appear to vary with the square root of the ionic strength, as was noted in an earlier study (17). Only at concentrations lower than 0.02 is change in free energy proportional to change in ionic strength.

The measurements upon egg albumin in 25 per cent ethanol at $-5°$C. are plotted in figure 9a. This method of plotting has the advantage that it presumably yields that part of the activity coefficient due to Coulomb forces that also obtains in aqueous solution. It is conceivable that measurements upon egg albumin and hemoglobin at a lower dielectric constant would reveal even greater changes in solubility with change in ionic strength, and that the maximum slope has not been attained even at $-5°$C. in 25 per cent ethanol. The solubility of egg albumin falls off very rapidly with further increase in per cent of ethanol and has therefore rendered difficult the carrying out of measurements in such solvents.

Comparison of the shapes of the curves (figure 9b) for proteins with those for glycine, its peptides, and the tetrapole lysylglutamic acid, suggests that the general phenomenon is the same for amino acids, peptides, and proteins. The far greater influence of salts upon the latter suggests that they have far greater dipolar and multipolar moments. Their far greater size presumably accounts for the very rapid falling off of protein solubility curves from their limiting slopes with increase in salt concentration, though the distribution of charged groups will also influence curvature.

The study of peptides (16) led to the conclusion "that, as a first approximation, the logarithms of the activity coefficients of dipolar ions increase as the concentration of the salt, and the dipole distance." The peptides thus far studied are, however, roughly rod-shaped, whereas glycine, cystine, egg albumin, and hemoglobin are more nearly spherical molecules.

The egg albumin molecule may be considered roughly spherical. Its viscosity coefficient is close to that demanded by Einstein's equation (39, 18; see also Polson (52)). Its radius estimated as a sphere is 21.7 A.U. Adding 1.23 A.U. for the radius of sodium chloride yields a value of a of approximately 23 A.U. Were it sufficient to use only the first term of Kirkwood's equation for a single dipole in the case of a molecule containing twenty-seven positive and twenty-seven negative charges on its surface, the limiting slope 14, estimated from the interaction with sodium chloride, would give a value of 52 A.U. for R, or only slightly greater than the diameter of this protein (27).

Hemoglobin is not quite spherical according to Svedberg (58). The radius estimated by Einstein's law is 34 A.U., or considerably larger than

the 27 A.U. estimated on the assumption of a spherical molecule. Hemoglobin may thus be estimated to have a value of a between 28 and 35 A.U. Carrying out the same calculation on the basis of a limiting slope of 17 yields a value for R of from 62 to 69 A.U. In the case of this protein, as of egg albumin, the dipole distance estimated in this way is thus not far greater than the diameter of the molecule, and smaller even than if two parallel dipoles were at opposite edges of the molecule, although hemoglobin presumably has at least seventy-five dipole pairs.

The problem of the electrostatic forces between ions and such multipoles as the proteins has been considered by Kirkwood (35), who has tentatively concluded that they should not be treated as single dipoles. The first term of his equation for a spherical molecule should therefore not suffice, for it will give far too low an estimate of the summed electrical moments. The problem is further complicated in the case of the protein, since the charged groups are probably situated near the edge of the molecule. For glycine the diameter of the molecule is estimated to be 5.64 A.U. (13) and the dipole distance (34) 3.17 A.U. The difference, 2.47 A.U., may be considered the distance of the charged groups from the edge of the glycine molecule. Assuming the charged groups to be the same distance from the edge of protein molecules gives the maximum distance for a single dipole as 41 A.U. for egg albumin. Considering not only the limiting slope, but also the shape of the solubility curve (35), suggests that it is not the dipole, but the quadrupole and octopole moments that dominate the interaction of egg albumin with salt. Although studies of this kind should aid in our analysis of structure, the large number of charges involved preclude a unique solution of the distribution of the charges on the surface of the vast protein molecule.

Relation between dielectric constant increments of dipolar ions and their interaction with salts

There is as yet no satisfactory theory relating the dipole moments of polar molecules and the dielectric constants of their solutions. The present state of knowledge regarding this problem has been discussed in detail by others in this symposium, and will not therefore be considered here. (See Onsager (49) and Wyman (65).) The estimation of the electric moments of dipolar ions by their study in non-polar solvents has thus far not been possible. The molecules studied were insoluble in such solvents. Moreover they might be expected to pass over into their uncharged isomers under these conditions.

A modification of the classical theory for the case of polar solvents has recently been suggested by Wyman (64), according to which the dielectric constant increment per mole of solute is a nearly linear expression of the

polarization of solute molecules. According to it, and according to Debye's (20) relation between electric moment and polarization, δ is linear, not in the dipole moment but in its square. Werner Kuhn and Hans Martin (37) have also considered the problem of the shape of dipolar ions as revealed by dielectric constant measurements, and concluded that: "The electric moment and therefore the distance between the end groups of the molecules increase proportionately with the square root of the number of members in the chain . . . . on the basis of statistical considerations" (37, p. 1528).

There is reason to believe that for dipolar ions of the same electric moment that part of the interaction with electrolytes which depends upon Coulomb forces is closely the same, regardless of the size of the molecule. Thus the solvent action of small concentrations of lithium chloride is closely the same for glycine and leucine in 90 per cent ethanol (10), whereas glycine is dissolved by low concentrations of sodium chloride in aqueous solution, and leucine, by virtue of its long paraffin chain, is salted out under the same conditions (51).

Not only is the interaction between dipolar ions and electrolytes for low concentrations of salt in regions of low dielectric constant closely the same for molecules having the same dipole distance, but the limiting slope,

$$[(D/D_0) \log N/N']/(D_0/D)\ \Gamma/2 = KR'$$

is greater the greater the dipole distance. This is readily demonstrated if we tentatively consider the square root of the dielectric constant increment proportional to the dipole distance and assume R for glycine to be 3.17 A.U. The quantity $\sqrt{\delta/2.3}$ then yields an estimate of the dipole distance R of molecules of known dielectric constant increment. The assumptions underlying this very rough approximation have been considered elsewhere in detail (11). The usefulness of the generalization and the close parallelism between the limiting slope and this quantity are given by the data in table 5. For the peptides studied the limiting slopes are approximately equal to $0.1\ \sqrt{\delta/2.3}$. Although this relation does not hold in this form for the proteins that have been thus far investigated by these two methods, there is no doubt that it is the distribution of charges and the effective electrical moments of molecules that determine their influence both on the interaction between ions and dipolar ions and on the dielectric constant of solutions.

To the technical difficulties of measuring, and the theoretical difficulties of interpreting, the dielectric constant of solutions of other polar molecules, must be added, in the case of the proteins, the difficulty of employing sufficiently long wave lengths to overcome the anomalous dispersion due to these enormous molecules and of obtaining solutions of sufficiently low

conductivity. Indeed, earlier measurements suggested that solutions containing proteins had lower dielectric constants than water.

The difficulties of achieving conditions under which satisfactory measurements of the dispersion of proteins may be made have by now been largely overcome. Egg albumin and hemoglobin, as well as zein (62, 63), have been studied in more than one laboratory and the results, though not yet entirely concordant, are of the same order of magnitude and leave no doubt as to the approximate influence of proteins on the dielectric constant of solutions.

TABLE 5

Molecular volumes, number of dipole pairs, influence on dielectric constant, and interaction with salts of certain amino acids, peptides, and proteins

SUBSTANCE	MOLECULAR WEIGHT M	MOLECULAR VOLUME V	RADII OF SPHERICAL MOLECULES b	NUMBER OF DIPOLE PAIRS	LIMITING SLOPE $\left(\frac{(D/D_0)\log N/N'}{(D_0/D)C}\right)$ KR'	$\sqrt{\frac{\delta}{2.3}}$
Amino acids						
Glycine	75.05	57.0	2.82	1	0.32	3.17
Leucine	131.10	122.2	3.63	1	0.30	3.17
Cystine	240.20	156.1	3.94	2	0.42‡	†
Peptides						
Diglycine	132.07	93.3	4.00*	1	0.58	5.51
Triglycine	189.11	129.6	5.75*	1	0.8	7.02
Lysylglutamic acid	275.20	211.5	7.12*	2	1.2	12.25
Proteins						
Egg albumin	34,000	25,500	21.7	27	14	42 65
Hemoglobin (horse)	66,700	50,000	27.0	75	17	114

* One-half the lengths of these rod-shaped molecules, considered as cylinders.

† Too insoluble to study by this method.

‡ See footnote 7 and equation 8.

Errera (6, 24) reported measurements of the frequency variation of the dielectric constant for dilute aqueous solutions of several proteins, including egg albumin and hemoglobin. He estimated very low dielectric constants of between 4 and 5 for proteins in the solid state and dielectric constants of their aqueous solutions which yield values of δ of approximately 10,000 for egg albumin and 30,000 for hemoglobin.

The dielectric constants of aqueous solutions of isoelectric egg albumin have also been measured by Shutt (56) at 18°C. and at a frequency of 110 cycles per second. His results yield a value of approximately 4000. On-

cley (48), working in our laboratory on the same preparations of egg albumin and hemoglobin that were employed in the solubility studies, has essentially confirmed Shutt's values for δ for egg albumin and Errera's values for hemoglobin. Moreover, his results yield essentially the same estimates for the relaxation times of both proteins as those obtained by Errera. Measurements of the relaxation times of the same proteins by the methods of Malsch (40) and Debye (21) are being made simultaneously. The study of proteins by these diverse methods should result in accurate information regarding their relation to, and influence on, the dielectric constant.

Assuming that the dielectric constant increments for proteins also increase as the square of the mean dipole moment, and estimating R as the square root of $\delta/2.3$ yields 42 A.U. for egg albumin on the basis of Shutt's measurements and 65 on the basis of Errera's. Errera's value of 30,000 for δ leads to an estimate of 114 for the R of hemoglobin. The electric moments suggested by these estimates thus range from 200 to 550 $\times 10^{-18}$ E.S.U. These calculations suggest dipole moments which are of the same order as would result from single dipoles, or, at most, double dipoles situated at the opposite edge of these proteins. These results are thus consistent with those deduced from our solubility measurements, and suggest that the distribution of the charges on the surface of these proteins is such that the resultant moments are far smaller than they would be were all the dipole pairs on opposite sides of the molecule. None the less, these moments are far greater than those of any chemically well defined molecules that have thus far been investigated. It is largely as a result of these considerations that we have investigated the interactions of dipolar ions, and of ions with dipolar ions, in regions of high dielectric constant.

III. CHANGE IN FREE ENERGY IN SYSTEMS OF HIGH DIELECTRIC CONSTANT

In regions of high dielectric constant the interaction between ions and dipolar ions cannot be entirely described in terms of Coulomb forces. It is under these conditions that the principle of the ionic strength does not suffice to define the change in free energy of a dipolar ion even in dilute salt solutions, and that the specific properties of both ions and dipolar ions must be considered.

Systems containing more than one dipolar ion

Proteins, as well as phospholipoids, amino acids, and peptides, must ultimately be studied in the presence of one another if we are to understand the conditions that obtain in biochemical systems. The principles that have thus far been established are illustrated by the solubility of cystine in aqueous solutions of urea, amino acids, and the peptide diglycine.

The solvent action of neutral salts upon cystine has already been considered (figure 5). Glycine increases the solubility of cystine approximately to the same extent as sodium chloride (figure 10), although the solvent action in the case of the dipolar ion is smaller at low concentration and greater at high concentration.

At the same molar concentration α-aminobutyric acid has a much smaller influence than glycine in diminishing the activity coefficient—increasing the solubility—of cystine. It follows that in the interaction between dipolar ions not only their influence on the dielectric constant of solutions, but their specific chemical groups must be considered. The longer the paraffin chain, the smaller the influence of dipolar ions of the same electric moment in decreasing the activity coefficient of other dipolar ions, as of ions (26, 33). Moreover, diglycine, which has a larger dipole moment and

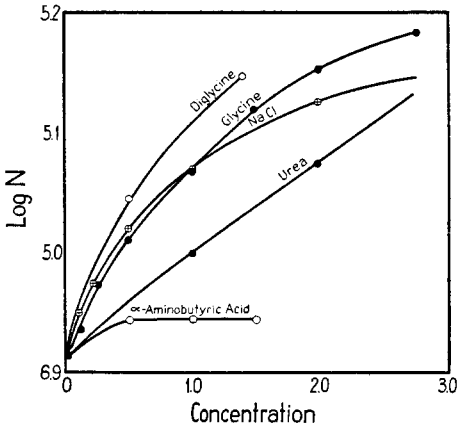


FIG. 10. Influence of various substances on solubility of cystine

in which all non-polar groups are between the charged ammonium and carboxyl groups (42), has a greater influence per mole than glycine, though a smaller influence than would be expected if the interaction of dipolar ions depended only on the dielectric constant of the solutions.

The solubility curves for cystine in glycine, diglycine, and α-aminobutyric acid would appear to belong to the same family, whereas the logarithm of the solubility of cystine is almost linear in the concentration of urea, suggesting a somewhat different mode of interaction between dipolar ions than between amino acids and urea. Although urea solutions have higher dielectric constants than water, the dielectric constant of even a saturated aqueous solution of urea is less than 100, or smaller than that of a molal solution of glycine, though the solvent action of this small polar molecule is far greater on both amino acids and proteins.

Systems containing concentrated electrolyte and more than one dipolar ion

If we now turn to systems containing three components—sodium chloride, cystine, and another dipolar ion—we have the changes in activity coefficient of the cystine indicated in figure 11. The solubility of cystine is changed far less by salt in systems containing amino acids or peptides than in aqueous solutions, or in solutions of still lower dielectric constant. The higher the concentration of the amino acid, the smaller the change in solubility due to the neutral salt. This might have been anticipated, since electrostatic forces are smaller the higher the dielectric constant.

Excepting in dilute salt solution the interaction with cystine varies considerably, depending on the nature of the dipolar ion employed in increas-

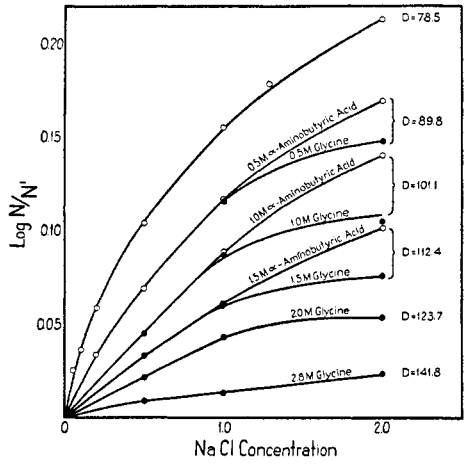


FIG. 11. Influence of amino acids on interaction between sodium chloride and cystine

ing the dielectric constant. Sodium chloride changes the free energy of cystine more in α-aminobutyric acid than in glycine solutions. This observation is related to two others. In the first place the activity coefficient of cystine is, as we have seen, decreased far more by glycine than by α-aminobutyric acid. In the second place, amino acids of longer hydrocarbon chain have a greater effect in increasing the activity coefficients of salts in concentrated salt solutions.

The influence of amino acids on sodium chloride has been estimated by the use of amalgam electrodes in cells without liquid junction (33). In sodium chloride solutions more concentrated than 1 molal, change in free energy of the electrolyte in the presence of amino acids has been shown to be approximately independent of electrolyte concentration, and the relations shown in table 6 obtain. The changes in the logarithm of the

activity coefficient of the amino acids (component 2) due to the salt (component 3) are calculated from E.M.F. measurements, according to the relation of Bjerrum (4).

$$\frac{\delta \log f_2}{m_3} = \upsilon \frac{\delta \log f_3}{m_2} \tag{7}$$

The results of this calculation yield values for the salting-out constants, Ks, of leucine in sodium chloride, in good agreement with that estimated from the solubility measurements of Pfeiffer and Würgler (51) as 0.09. The value of Ks_{NaCl} for α-aminobutyric acid we have determined to be 0.04 or just double the value, as deduced by equation 7, for the effect of the amino acid on the salt (33). Positive in sign, these activity coefficients indicate salting-out of the salt by the amino acid, and of the amino acid by the salt.

TABLE 6

Activity coefficients of amino acids and sodium chloride in concentrated salt solution

AMINO ACID	CHANGE IN LOG f OF NaCl DUE TO AMINO ACID. OBSERVED BY E.M.F. MEASUREMENTS	CHANGE IN LOG f OF AMINO ACID DUE TO NaCl. CALCULATED FROM E.M.F. MEASUREMENTS	CHANGE IN LOG f OF AMINO ACID DUE TO NaCl. OBSERVED BY SOLUBILITY MEASUREMENTS
Glycine	0.01	0.02	
α-Alanine	0.01	0.02	
α-Aminobutyric acid	0.018	0.035	0.04
Valine	0.045	0.09	
Leucine	0.06	0.12	0.09

Systems containing dilute electrolyte and more than one dipolar ion

Interaction between electrolytes and dipolar ions takes place in biological systems at an ionic strength of approximately 0.16. The measurements reported in figure 11 indicate that at such salt concentrations glycine and α-aminobutyric acid have closely the same effect on the interaction between cystine and sodium chloride. This result suggests that *in sufficiently dilute salt solution the predominant effect of dipolar ions depends upon their influence on the dielectric constant of solutions and is largely independent of the nature of the dipolar ion.* Studies comparable to these have also been carried out in aqueous solutions of urea and diglycine.

Whereas decrease in activity coefficient due to Coulomb forces varies inversely as the second power of the dielectric constant, the increase in activity coefficient characteristic of both amino acids and proteins, and known as the salting-out effect, would appear to vary far less with temperature or dielectric constant. Any expression characterizing the behavior

of amino acids and proteins at high dielectric constant, even in dilute salt solution, must therefore contain a term opposite in sign from that due to Coulomb forces. For the case of cystine in systems containing sodium chloride and glycine, we may write for the limiting slope:

$$\frac{D}{D_0} \log \frac{N}{N'} = \left[KR - \frac{D}{D_0} Ks \right] \frac{D_0}{D} \frac{\Gamma}{2} = \left[0.56 - \frac{D}{D_0} 0.14 \right] \frac{D_0}{D} \frac{\Gamma}{2} \qquad (8)$$

The value of Ks increases among dipolar ions of the same electric moment with the length of the paraffin side chain, whereas Coulomb forces, as we have seen, largely depend upon the dipole moment and only to a much smaller extent upon the radius of a molecule. The larger the number of non-polar groups in comparison with the dipole moment, the lower the dielectric constant must therefore be in order that the change in free energy with change in ionic strength may yield an estimate of Coulomb forces. Conversely, the higher the dielectric constant the smaller the first term in such an expression becomes, and the more accurately Ks can be estimated.

The very small solvent action of low concentrations of sodium chloride upon cystine is approximately accounted for by assuming the value 0.14 for Ks of cystine in such systems. This value for this constant may also be calculated by means of an approximate expression of Kirkwood's (35; and see equation 28 in reference 11) developed for spherical molecules without distinguishing in its tentative form between dipolar ions with paraffin chains and those in which the CH_2 groups are largely between polar groups. It yields somewhat lower values for tyrosine and higher values for glycine and most proteins than those observed in concentrated salt solutions.[11]

The salting-out effect may be considered as due at least in part to the fact that a dipolar ion "displaces a certain quantity of solvent and therefore reduces the polarization of the solvent by the salt ion" (35). The studies that have thus far been carried out in media of high dielectric constant indicate that such polarization effects will play a large rôle even in dilute salt solutions. Under these circumstances differences in the polarizability due to different ions will manifest themselves in biological systems, as well as differences due to the non-polar groups and dipole moments of

[11] Values of Ks measured in concentrated salt solutions would appear to be lower than those estimated as the difference between measurements in dilute salt solution and the expectation from Coulomb forces. In comparison with the value 0.14, given above, Ks of cystine in concentrated ammonium sulfate solution (figure 5) is 0.05. Comparably, the limiting slope determined for glycine in dilute aqueous sodium chloride by E.M.F. measurements is 0.24 (33), or approximately 0.08 less than in media of low dielectric constant, in good agreement with the value 0.06 calculated by Kirkwood's equation (35).

dipolar ions. The forces concerned would appear, however, not to be very different from those considered by Debye (19, 22) and others (31, 53) for the change in free energy of electrolytes in the presence of other electrolytes and non-electrolytes. Systematic studies may be expected to analyze in these terms also the interactions between salts, amino acids, and proteins in regions of high dielectric constant.

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