be expected to have moments close to that of diglycine. Their heptyl esters have therefore been prepared and measured in benzene and found to have moments of 3.6 and 3.5 $\times 10^{-18}$ e. s. u., respectively.[20] The moments of these larger molecules in benzene fall off rapidly with concentration. They suggest a value of 1.4 or 1.5 $\times 10^{-18}$ e. s. u. for the peptide linkage, whereas the dipole moment of the amide group has been estimated to be 3.2[21] in the gaseous state and 3.6 to 3.8 in nonpolar solvents.[22,23] The solvent action of neutral salts upon the hydantoic acids studied also suggests higher moments than are indicated by the measurements in benzene.

The moments of the esters of amino acids, peptides and of their hydantoic acids are, however, small in comparison with the superimposed effects due to dipolar ionization, and it is for the most part the latter which are responsible for the very large activity coefficients of peptides in salt solutions in regions of low dielectric constant. In regions of high dielectric constants, where the solubilities of amino acids and peptides are relatively high, the same contributions of Coulomb forces to activity coefficients presumably obtain, but are there supplemented by forces other than those due to the purely electrostatic interaction of ions and dipolar ions.

(20) We are indebted to Dr. Wyman for making these measurements.

(21) Zahn, *Trans. Faraday Soc.*, **30**, 804 (1934).

(22) Devoto, *Gazz. chim. ital.*, **63**, 491 (1933).

(23) Kumler and Porter, THIS JOURNAL, **56**, 2549 (1934).

## Summary

1. The interaction between ions and dipolar ions has been studied in regions of low dielectric constant in order to estimate the electrostatic forces involved.

2. Solutions of sodium chloride in 80% ethanol at 25° were employed as solvents, and glycine, diglycine, triglycine and the tetrapole lysylglutamic acid as solutes.

3. Hydantoic acids prepared from glycine and diglycine were studied in the same solvents in order to estimate what proportion of the effect was due to the dipolar ion structure of amino acids and peptides and what to the polar groups of these molecules.

4. The solvent action of neutral salts upon amino acids and peptides in dilute solution appears, as a first approximation, to be a function of their dipole moments.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

# Studies in the Physical Chemistry of the Proteins. XIII. The Solvent Action of Sodium Chloride on Egg Albumin in 25% Ethanol at −5°

BY RONALD M. FERRY, EDWIN J. COHN AND ETHEL S. NEWMAN

**Introduction.**—The relation of the properties of amino acids and peptides to their chemical structure, and to the number and distribution of the electrostatic charges which they bear, has been considered in recent investigations.[1-3] Even when they have no net charge, these molecules, by virtue of their dissociated ammonium and carboxyl groups, have large electric moments and contribute markedly to the dielectric constants of their aqueous solutions, thereby diminishing electrostatic forces. The solubility of these dipolar ions diminishes, however, with diminution of the dielectric constant of the solvent, and under these conditions the properties of the solutions approach those of the pure solvents, and the effects of electrostatic forces are more readily observed.

It is of interest to extend these investigations to more complex dipolar and multipolar ions.[4] We have, accordingly, studied the solubility of isoelectric egg albumin in 25% ethanol–water mixtures at −5°. Egg albumin is a well-defined, easily purified, protein. Its molecules are roughly spherical with a radius of about 22 Å.[5] Early estimates revealed twenty-seven dissociable acids and the same number of dissociable basic groups,[6] whereas a recent study suggests a slightly higher number of dissociable groups.[7] Albumins are de-

(1) Cohn, *Naturwissenschaften*, **20**, 663 (1932).

(2) Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934).

(3) Cohn, McMeekin, Greenstein and Weare, THIS JOURNAL, **58**, 2365 (1936).

(4) Such amino acids as cystine, such peptides as lysylglutamic acid, and all proteins are in reality multipoles. None the less they have an effective resultant dipole moment.

(5) Svedberg, *Kolloid Z.*, **51**, 10 (1930).

(6) Cohn, *Physiol. Rev.*, **5**, 349 (1925).

(7) Cannan, *Biochem. J.*, **30**, 227 (1936).

fined as proteins "soluble in pure water and coagulable by heat." Aqueous solutions containing 40% of egg albumin are readily prepared, but in the experiments reported in 25% ethanol the solubility of egg albumin was only 0.13 g. per liter, and neutral salts increased its solubility many fold, as they do with globulins in aqueous solution.

Although knowledge of their chemical structure is incomplete, it appears possible from this study and those subsequently to be reported that proteins may be completely characterized in terms of their size, shape, the length of their paraffin side chains, and the number and distribution of their polar, and especially of their charged, groups.

**Methods and Materials.**—The egg albumin used in these experiments was crystallized five times from ammonium sulfate solutions according to the procedure described by Sørensen.[8] Subsequently it was dialyzed in cellophane membranes against distilled water until the dialysate gave a negative sulfate test. In our early experiments it was then concentrated in negative pressure dialyzers[8] and dialysis continued until heat coagulated albumin yielded no sulfate or chloride to large volumes of hot water, even after its concentration from a volume of about 300 to 1–2 cc.

Our early experiments suggested that the saturating body contained traces of protein salt as well as uncombined isoelectric protein. Electrodialysis was therefore also used. The material obtained after dialysis in cellophane membranes was placed in a Pauli electrodialysis apparatus with a potential of 110 volts between electrodes. The contents of the cell were stirred at intervals, and dialysis continued against conductivity water, until the current passing through the cell became constant. A very small amount of insoluble material often appeared during this process and was filtered off. The soluble material was readily crystallizable.

After electrodialysis the egg albumin was concentrated in negative pressure dialyzers as before, where it reached a concentration of about 40% by weight. It was next dried according to the method of Adair[9] at about 2° in a vacuum over phosphorus pentoxide. The dry material was ground to a fine powder in a porcelain mortar and stored in vacuum desiccators over phosphorus pentoxide. It was readily soluble and crystallizable from ammonium sulfate solutions. Of the crystal forms figured by Sørensen and Hoyrup[10] the rhombic plates, rather than the needles, were most often observed.

The solvents employed were made up to contain 25% ethanol by volume at 20° and varying amounts of C. P. sodium chloride. The systems containing most salt therefore contained slightly less water. It may ultimately be desirable to calculate solubilities in systems having the same ethanol–water composition.[3]

The thermostat employed was maintained at −5 ± 0.05°, and contained kerosene. Weighed amounts of dry powdered egg albumin were placed in double stoppered Pyrex centrifuge bottles. One of these is shown in Fig. 1. These were placed in a holder in the bath and completely filled with solvent which already had been cooled to −5°. The outer caps (B) were well greased, but not the inner ground glass stoppers (A). The bottles were slowly rotated in a horizontal position until equilibrium was attained.

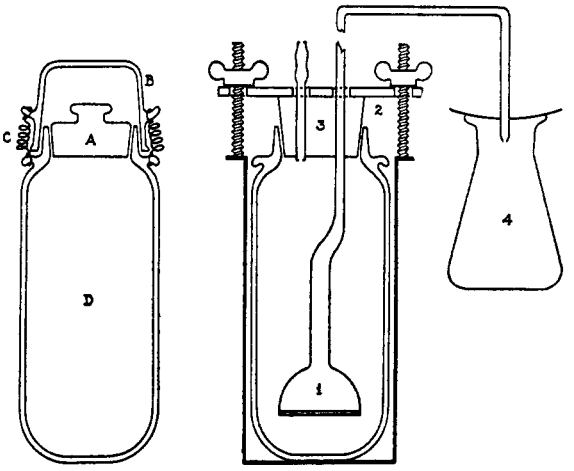


Fig. 1.—A, ground glass stopper; B, outer ground cap; C, springs; D, Pyrex bottle; 1, sintered filter; 2, yoke to hold stopper; 3, rubber stopper; 4, weighing bottle.

Although ninety hours of rotation apparently sufficed for saturation, rotation was continued for five to seven days. At the end of this time, rotation was stopped, the bottles were placed in a vertical position, and undissolved egg albumin allowed to settle. Sintered Jena glass filters (porosity G 4)—previously cooled to −5°—were then introduced into the equilibrium bottles without removing the latter from the bath (see Fig. 1). The supernatant fluid was filtered under 15 lb. (1 atm.) pressure of nitrogen gas into glass-stoppered weighing bottles, and the content of protein in the weighed sample determined.

After removing the supernatant fluid as completely as possible from the equilibrium bottles, they were again filled with solvent at −5° and again equilibrated. Equilibrium was always approached by the saturation of solvents, the operation often being repeated as many as twelve times with a single sample of egg albumin. Even at the end of months of rotation in the thermostat by far the greater part of the undissolved material remained soluble and crystallizable. The small amount of denatured protein had no marked influence on these experiments. The solubility of egg albumin, denatured in 25% ethanol at room temperature, did not exceed 0.004 g. for 1000 g. solution under the conditions of our experiments, a quantity small in comparison with the solubilities here reported. Moreover, under these conditions, no solvent action of salt on the denatured egg albumin was detected.

Two different analytic procedures were employed. In case the solvent contained no salt, egg albumin was estimated gravimetrically. Weighed aliquots were dried to constant weight in an oven at 95°.

In the case of solutions containing salt the alcohol was first evaporated. A few cubic centimeters of acetate buffer of *p*H 4.85 and about 0.5 g. of potassium sulfate were

(8) Sørensen, *Compt. rend. trav. lab. Carlsberg*, **12**, 1 (1917).

(9) Adair, *J. Physiol.*, **72**, 2P (1931).

(10) Sørensen and Hoyrup, *Compt. rend. trav. lab. Carlsberg*, **12**, 164 (1917).

then added in order to ensure complete coagulation of the protein over the steam-bath. The coagulated protein was transferred quantitatively to weighed sintered glass crucibles, of porosity G4, and washed until the filtrate gave a negative sulfate test. The crucibles and contents were dried to constant weight at 95°. The results obtained accorded satisfactorily with those based on Kjeldahl nitrogen analysis. Aliquots containing from 0.02 to 0.6 g. of dry protein were analyzed. Duplicate analyses accord within 0.3 to 0.4 mg. The errors due to change in weight of crucibles and losses in transfer did not exceed 0.5 mg., or at most 3%. Other factors not so well defined, such as variation in *p*H, presence of traces of protein salt, etc., gave rise to larger discrepancies and will be discussed subsequently. Change in the apparent dissociation constants of buffer acids in alcohol rendered their use undesirable as solvents in these investigations. The *p*H of each solution was, however, colorimetrically estimated at room temperature against standard buffers—0.05 *N* in acetate—in 25% ethanol.[11] The apparent isoelectric point of egg albumin in 25% ethanol is presumably increased by something less than one *p*H unit, the estimated values being more acid the larger the concentration of salt and protein in the systems.

**Solubility Measurements.**—The solubility of the first preparation studied diminished with each successive equilibration with solvent. An experiment of this kind is reported in Table I. Often more than a month would elapse until solubility became constant in successive solvents. Since the saturating body always contained egg albumin, which was both soluble and crystallizable, the slow diminution in solubility was believed to be due to the washing out of a more soluble impurity, and not to denaturation of the protein. Accordingly electrodialysis was resorted to in order to reduce the conductivity of the preparations as far as possible, even at the risk of denaturating some of the egg albumin. The electrodialyzed material generally exhibited constant solubility, as is indicated by the experiment reported in Table I. Thus prepared egg albumin had from the beginning the same solubility as was ultimately reached by the earlier preparation.

TABLE I

COMPARISON OF DIALYZED AND ELECTRODIALYZED EGG ALBUMIN IN 25% ETHANOL CONTAINING 0.005 *N* NaCl AT −5°

| Hours of equilibration | Estimated *p*H | Soly., g./1000 g. soln. |
|---|---|---|
| Preparation CVB | | |
| 89 | 5.63 | 0.358 |
| 93 | 5.69 | .269 |
| 112 | 5.75 | .234 |
| 212 | 5.80 | .209 |
| 259 | 5.87 | .228 |
| 162 | 5.67 | .268 |
| 498 | 5.70 | .253 |
| 185 | 5.74 | .166 |
| 233 | 5.74 | .175 |
| 282 | 5.72 | .165 |
| Preparation CVI, electrodialyzed | | |
| 138 | 5.68 | 0.157 |
| 210 | 5.90 | .173 |
| 191 | 5.86 | .157 |
| 209 | 5.86 | .175 |
| 188 | 5.84 | .151 |
| 254 | | .174 |

(11) Michaelis and Mizutani, *Z. physik. Chem.*, **116**, 135 (1925).

In Table II are reported solubility measurements upon four egg albumin preparations, the last of which, on which most measurements were

TABLE II

THE INFLUENCE OF NaCl ON THE SOLUBILITY OF EGG ALBUMIN IN 25% ETHANOL AT −5°

| Concn. of NaCl moles/liter | Egg Albumin Preparation CVB | CVA | $CVB_2$ | CVI[a] | Average |
|---|---|---|---|---|---|
| | Solubility: g. per 1000 g. of solvent | | | | |
| 0.0 | 0.119 | | 0.109 | 0.151 | |
| | .129 | | .147 | .123 | |
| | .118 | | .112 | .114 | |
| | | | .148 | .128 | |
| | | | | .138 | |
| | | | | .149 | |
| | | | | ,109 | |
| | | | | (.063) | |
| | | | | .110 | 0.13 |
| .00505 | .166 | | .174 | .157 | |
| | .175 | | .146 | .173 | |
| | .165 | | (.210) | .157 | |
| | | | .150 | .175 | |
| | | | .151 | .151 | |
| | | | | .174 | |
| | | | | (.125) | .16 |
| .0101 | .224 | | .200 | .203 | |
| | .222 | | .153 | .199 | |
| | .222 | | (.128) | (.324) | |
| | | | .194 | .225 | |
| | | | .221 | .176 | .20 |
| .0202 | | | | .292 | |
| | | | | .293 | |
| | | | | .244 | |
| | | | | .223 | |
| | | | | .243 | |
| | | | | .233 | .26 |
| .0505 | | 0.527 | .569 | (.649) | |
| | | .504 | .523 | .519 | |
| | | | .497 | (.684) | |
| | | | | .509 | |
| | | | | (.632) | |
| | | | | .556 | |
| | | | | .535 | |
| | | | | .461 | .52 |
| .101 | | | | 1.32 | |
| | | | | 1.04 | |
| | | | | 1.20 | |
| | | | | 1.28 | 1.2 |
| .201 | | | | 4.11 | |
| | | | | 4.10 | 4.1 |
| .349 | | | | 18.5 | |
| | | | | 18.2 | 18.4 |
| .489 | | | | 38.9 | |
| | | | | 43.8 | 41.4 |

[a] Electrodialyzed.

made, was electrodialyzed. In the case of the earlier series in which non-electrodialyzed material was used we have tabulated only those values which indicated that a reasonably constant solubility had been attained. In later experiments with electrodialyzed material at low salt concentrations a few points, which were clearly discordant, are given in parentheses and not included in the averages. These were probably due to temporary failure in the thermoregulator, a source of trouble only recently overcome by removing the relay from the cold room. At the highest salt concentrations only the first value for solubility is given, since at such high solubilities it was difficult to provide sufficient saturating body for repeated equilibration.

The deviations are far greater than in most studies from the laboratory, because of the difficulty of achieving equilibrium and of controlling small deviations in *p*H and in alcohol concentration. None the less, because of the very large solvent action at low sodium chloride concentrations, these determinations yield a very satisfactory estimate of the influence of salt on the solubility of egg albumin. Average values of solubility, expressed as grams per 1000 g. of solution, are given in the last column of Table II. Taking the density of the solvents as given by the approximate relation

$$\rho = 0.9761 + 0.043\ C$$

and the apparent specific volume of egg albumin as 0.75[12] and its molecular weight as 34,000, its solubility has been computed as moles per liter at −5° and mole fraction in Table III.

TABLE III

THE INFLUENCE OF NaCl ON THE SOLUBILITY OF EGG ALBUMIN IN 25% ETHANOL AT −5°

| Concn. of NaCl $C$ | $(D_0/D)C$ −5° | *p*H at 20°C. | Soly. of egg albumin Moles × $10^5$ per liter | Mole fraction $N \times 10^7$ | $(D/D_0)$ log $N/N'$ |
|---|---|---|---|---|---|
| 0.0 | 0.00 | 5.9 | 0.374 | 0.788 | 0.00 |
| .00505 | .0058 | 5.8 | .460 | .969 | .08 |
| .0101 | .0116 | 5.7 | .573 | 1.21 | .16 |
| .0202 | .0231 | ... | .745 | 1.57 | .26 |
| .0505 | .0578 | 5.6 | 1.49 | 3.15 | .53 |
| .101 | .116 | 5.5 | 3.44 | 7.26 | .84 |
| .201 | .230 | 5.4 | 11.9 | 25.3 | 1.31 |
| .349 | .399 | 5.3 | 53.7 | 115.0 | 1.89 |
| .489 | .559 | 5.3 | 122.0 | 266.0 | 2.21 |

**Results.**—The influence of neutral salts on each other increases with the square root of the ionic strength of the solvent, and with the valence type of the salt.[13,14] A tenth molal solution of potassium nitrate increases the solubility of thallous chloride approximately 20%,[15] of barium iodate 80%.[16] The solubility of luteo-hexacyanocobaltiate, a tri-trivalent electrolyte studied by Brönsted and Peterson,[17] is increased over ninefold by 0.1 mole of potassium chloride. Only an electrolyte of such high valence type approximates the behavior of proteins[18] at finite concentration.

The influence of neutral salts upon dipolar ions is not identical with that upon ions. Dipolar ions in the isoelectric condition have no net charge. In an extension of Debye's theory to the interaction between ions and dipolar ions, Scatchard and Kirkwood[19] have shown that the first term in the equation for the logarithm of the solubility ratio is proportional to the ionic strength, and not to its square root. The results upon egg albumin reported here are consistent with this prediction.

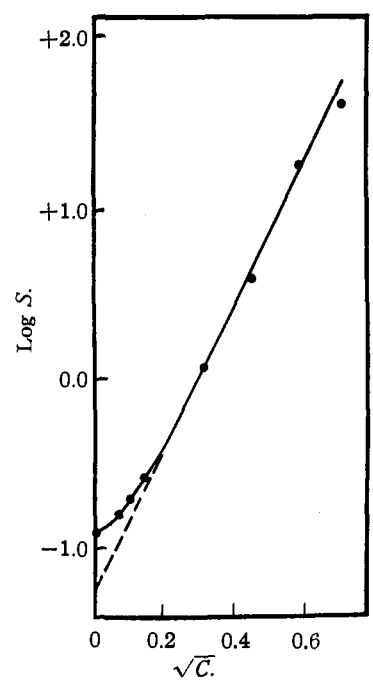


Fig. 2.—The influence of NaCl on the solubility of egg albumin in 25% ethanol at −5°.

In Fig. 2 the logarithm of the solubility is plotted against the square root of the concentration of

(12) Svedberg and Nichols, THIS JOURNAL, **48**, 3081 (1926).

(13) Debye and Hückel, *Physik. Z.*, **24**, 185 (1923).
(14) Debye, *ibid.*, **25**, 97 (1924).
(15) Bray and Winninghoff, THIS JOURNAL, **33**, 1663 (1911).
(16) Harkins and Winninghoff, *ibid.*, **33**, 1827 (1911).
(17) Brönsted and Peterson, *ibid.*, **43**, 2265 (1921).
(18) Cohn and Prentiss, *J. Gen. Physiol.*, **8**, 619 (1927).
(19) Scatchard and Kirkwood, *Physik. Z.*, **33**, 297 (1932).

sodium chloride. The logarithm of solubility does not appear to be proportional to $\sqrt{C}$ when the latter is less than 0.1. In high concentrations of salt, where Debye's law for ions would not be expected to hold, however, the points so plotted apparently fall on a straight line as noted in an earlier study.[18]

Cystine, a tetrapole, is the least soluble of the naturally occurring amino acids. Its solubility also demonstrates that the logarithm of the activity coefficient is not linear in the square root, but is a function of the concentration (see Fig. 2, ref. 20).[20] Although the solubility of cystine is only increased 10% by 0.1 mole of sodium chloride, the same concentration of salt increases the solubility of egg albumin almost ten-fold.

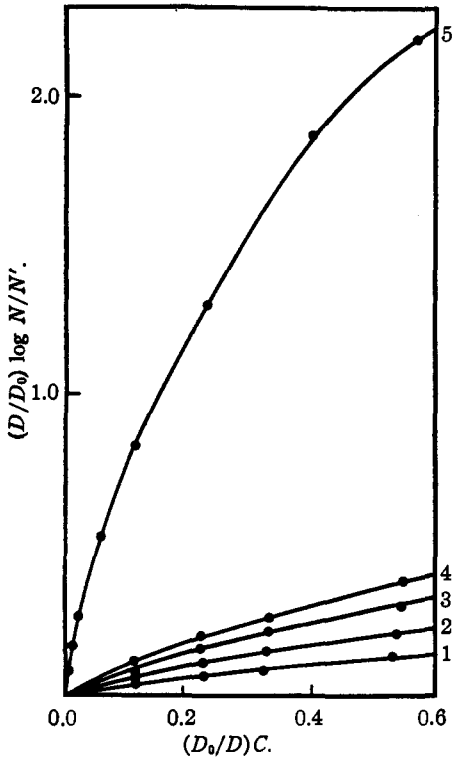


Fig. 3.—Solvent action of NaCl on: (1) glycine; (2) diglycine; (3) triglycine; (4) lysylglutamic acid and (5) egg albumin.

Many amino acids and peptides are so soluble in water, and increase the dielectric constant of the solutions to such an extent that electrostatic forces due to the interaction between ions and dipolar ions cannot be studied in this medium. They have accordingly been studied in alcohol–water mixtures in which their solubility was reduced and electrostatic forces due to this interaction increased. Under these circumstances it has been demonstrated for glycine that as a first approximation the logarithms of the solubility ratio increase as the second power of the reciprocal of the dielectric constant, as demanded if the effect is due primarily to Coulomb forces. Measurements made in from 60 to 95% ethanol, and plotted with $(D/D_0)$ log $N/N_0$ as ordinate, and $(D_0/D)\Gamma_2$—a quantity proportional to $\kappa^2$ in Debye's equation—as abscissa, fall on the same curve.[1,2]

The measurements on egg albumin in 25% ethanol are plotted in this manner in Fig. 3. This method of plotting has the advantage that it presumably yields that part of the activity coefficient due to Coulomb forces that also obtain in aqueous solution. The dielectric constant of 25% ethanol at $-5°$, obtained by interpolation from Wyman's data,[21] is taken as 78.6. That of water at $-5°$ was estimated by Wyman by extrapolation to be 90.0.[21] Accordingly the value 0.874 for $D/D_0$ has been employed tentatively in our calculations without any correction for the influence of salt or of egg albumin on the dielectric constant. It is conceivable that measurements upon egg albumin at still 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 in 25% ethanol at $-5°$. The solubility of egg albumin falls off so rapidly with further decrease in dielectric constant, however, that the accuracy of the measurements would be still further reduced, and it seemed preferable therefore to study another protein, hemoglobin, in a series of alcohol–water mixtures. This study will be reported subsequently.

The curve for egg albumin is compared in Fig. 3 with that of the peptides of glycine and with the tetrapole lysylglutamic acid that has been studied in 80% ethanol at 25°.[3] The solvent action of neutral salts on these molecules is in the order of their influence on the dielectric constant of solutions, and presumably of their electric moments. The shapes of the curves suggest that the general phenomenon is the same for amino acids, peptides and proteins, and the far greater influence of salts upon the latter suggests that they have far greater dipolar and multipolar moments. Their size is also far greater and this presumably accounts for the very rapid falling off of protein

(20) Cohn, "Annual Review of Biochemistry," Vol. IV, Stanford University, California, 1935, p. 93.

(21) Wyman, THIS JOURNAL, 53, 3292 (1931).

solubility curves from their limiting slopes with increase in salt concentration.

This phenomenon is more readily analyzed if we plot some function of change in solubility with change in concentration against some function of concentration. This has been done in Fig. 4. The forces which lead to the salting out of proteins are clearly manifest even in dilute solutions of protein and salt at low temperature. Whereas the measurements upon glycine fall on an essentially straight line, when plotted as in Fig. 4, and those upon lysylglutamic acid show some curvature; the curvature in the case of egg albumin is far greater.

There is no uncertainty in determining the limiting slope of glycine to be 0.30 to 0.32 by this method, or of lysylglutamic acid to be 1.1 to 1.2. The estimate for egg albumin, however, is not independent of the method of extrapolation. The average values of $(D/D_0)\log N/N'$ in Table III appear to be linear in $(D_0/D)C$ for values of the latter less than 0.02, and to yield 14 for the limiting slope.

The study of peptides[3] 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." Assuming the same rule to hold for egg albumin, and the dipole distance, $R$, of glycine[2] to be 3.17, would yield 140 Å. The peptides studied were, however, roughly rod shaped, whereas glycine and egg albumin are roughly spherical. Kirkwood has developed a theoretical equation for the activity coefficients of dipolar ions considered as spheres,[2] the first term of which gives the limiting slope for a single dipole as proportional to $R^2/a$. For egg albumin $a$, the sum of the radii of the protein and salt, may be taken as 23 Å. On this basis $R$ would be 52 Å. or only slightly greater than the diameter of the molecule. Although the large number of charges involved precludes a unique solution of the distribution of the charges on the surface of the vast protein molecule, a preliminary study[22] suggests that egg albumin should not be treated as a single dipole, but that the quadropole and octopole moments dominate the interaction with salts.

The influence of dipolar ions on the dielectric constant of solutions may also be considered, as a first approximation, a function of the electric moment, presumably of its second power.[23] In the case of peptides, plotting the limiting slope against the square root of the dielectric constant increment, $\delta$, yields a roughly linear relationship.[3] Errera[24] reports dielectric constants from which values of $\delta$ can be calculated of 10,000 and Shutt[25] of 4000. Assuming $\delta$ proportional to 2.3 $R^{2.20}$ yields values of $R$ of 42 Å. and 66 Å. on the basis, respectively, of Shutt's and Errera's dielectric constant measurements. This result is in as good agreement as can be expected with the value 52 Å. on the basis of our solubility studies and the first term of Kirkwood's equation. There is thus little doubt that it is the distribution of charges and the effective electrical moments of the molecule that determine both its influence on the dielectric constant of solutions and on the interaction with ions and other dipolar ions.

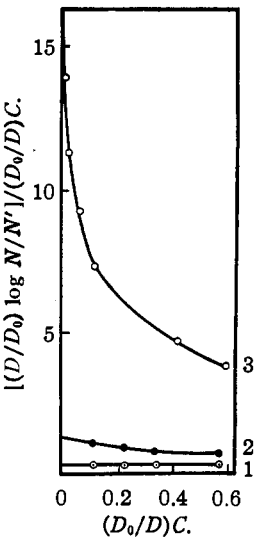


Fig. 4.—Diminution of solvent action of NaCl with increase in concentration upon: (1) glycine; (2) lysylglutamic acid; (3) egg albumin.

## Summary

1. The solubility of egg albumin has been studied in 25% ethanol at −5°. Under these conditions denaturation was slight and the solubility of the albumin so low, $3.7 \times 10^{-6}$ mole per liter, that its contribution to the dielectric constant of the solution was neglected.

2. Neutral salts increased the solubility of egg albumin under these conditions much as they do the solubility of globulins in water.

3. The solvent action of the neutral salt has been considered a measure of the electrostatic forces between the ions and the protein and compared with the comparable forces between ions, amino acids and peptides.

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(22) Kirkwood, personal communication. The analysis of these results in terms of this theory will be presented elsewhere.

(23) Wyman, THIS JOURNAL, **56**, 536 (1934).

(24) Errera, *J. Chim. Phys.*, **29**, 577 (1932).

(25) Shutt, *Trans. Faraday Soc.*, **30**, 893 (1934).