slightly smaller specific rotations, especially at the lower *p*H values.

The two stock solutions were prepared on separate occasions by dialysis of the same batch of ovalbumin (stored in the refrigerator as a paste in half-saturated ammonium sulfate). The results from one run were parallel to those from the other run, but the specific rotations differed by 1.60°. This difference may have been caused by the incomplete removal of the last traces of sulfate ions, since the *p*H values of the two stock solutions differed by 0.18 unit, and the *p*H values (4.62 and 4.80 in 0.15 *M* NaCl) were appreciably below the value for the isoionic protein reported by Cannan, Kibrick and Palmer[33] (4.95 and 4.97 in 0.133 *M* and 0.267 *M* KCl, respectively).

The results are plotted in Fig. 8 as specific rotation *vs.* number of protons dissociated. The observations appear to fall on a series of straight lines with markedly different slopes. The largest changes in slope occur at *p*H 4.0 and 5.8, but there is also an indication of a small change in slope at *p*H 11. The change in slope at *p*H 4.0 may be related to the change in slope that occurs in Fig. 1 at this *p*H. The change in slope at *p*H 5.8 does not, however, correspond to any break in the volume curve (Fig. 2). It takes place at almost exactly the point corresponding to the dissociation of the last of the 109 carboxyl groups of ovalbumin. If the changes in rotation between *p*H 5.3 and 6.8 reflect continuous changes in conformation with *p*H, then these same changes in conformation continue to take place above *p*H 6.8 and below 5.3, where the volume changes have more nearly their normal values. Therefore, if the abnormal volume changes observed with ovalbumin between *p*H 5.3 and 6.8 are caused by alterations in the conformation, these alterations are not reflected in the optical rotation.

According to Charlwood and Ens,[36] ovalbumin undergoes a small decrease in sedimentation constant below *p*H 4. The character of this change is, however, not the same as that observed with the optical rotation, since the sedimentation constant does not vary with *p*H except in the immediate vicinity of *p*H 4, whereas the optical rotation varies continuously with *p*H from *p*H 2 to about 12.

NOTE ADDED IN PROOF.—Dr. I. Klotz has made the pertinent suggestion that our volume changes may be influenced by changes in anion binding, especially in acid solutions. As Fig. 1 shows, however, the volume change per proton bound by ovalbumin is practically the same in 0.15 *M* NaCl as in the absence of salt. Furthermore, we have repeated some of the measurements shown in Fig. 2 without adding NaCl to any of the solutions. The observed points in these experiments fall on the same lines as those shown in Fig. 2. (Of course, the pH scale is not the same under these conditions.) Therefore it seems very unlikely that either anion or cation binding is a factor in these experiments. Kasarda[18] also has studied the effects of added electrolytes on the volume changes accompanying the titration of wool. He has found that 0.2 *M* NaCl does not have appreciable effects on these volume changes.

Dr. Klotz also has asked whether kerosene hydrocarbons might not be bound by our proteins. If such binding took place, and if it produced a volume change, time-dependent volume changes would have been observed both before and after mixing the solutions in the dilatometers because of the finite rate of diffusion of the hydrocarbons into the protein solutions. Furthermore our volume changes would have been much less reproducible than we have found them to be, since they certainly would have depended on the manner in which the acid and base solutions were mixed with the protein during a run. We have not seen any such effects in the experiments reported here for proteins, and we have, in fact, looked for them.

Acknowledgment.—This work was supported by a grant from the National Science Foundation.

(36) P. A. Charlwood and A. Ens, *Can. J. Chem.*, **35**, 99 (1957).

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Volume Changes in Protein Reactions. II. Comparison of Ionization Reactions in Proteins and Small Molecules

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RECEIVED NOVEMBER 13, 1961

The volume changes that accompany the reactions $R\text{-}COO^- + H^+ \rightarrow R\text{-}COOH$ (1) and $R_1R_2R_3NH^+ + OH^- \rightarrow R_1R_2R_3N + H_2O$ (2) have been measured dilatometrically for a number of acids and bases. As has been pointed out by Hammann and Lim, the variations in ΔV for reaction (1) when the group R is changed are considerably larger than the variations in ΔV for reaction 2 when R_1, R_2, and R_3 are changed. If the group R in reaction 1 bears a negative charge which is close to the carboxylate ion that binds the proton (as in oxalate$^{=}$, malonate$^{=}$ and maleate$^{=}$) the volume change of reaction 1 is considerably increased. If the group R contains a positive charge (as in glycine) the volume change of reaction 1 is decreased. Analogous effects of charges in the groups R_1, R_2 and R_3 are observed for reaction 2. These effects are qualitatively in agreement with the Drude–Nernst theory of ionic electrostriction, but the theory fails to give the observed dependence of volume change on ionic size. The relationships between the value of ΔV and the values of ΔF, ΔH, ΔS and ΔC_p for reactions 1 and 2 are discussed. They appear to be complex and suggest that ΔV provides a useful means of gaining insight into the structures of the hydration layers that surround organic anions and cations. The significance of the abnormally small volume changes observed for reaction 2 in proteins, as reported in Part I, is not clear. In none of the amines studied were values of ΔV observed that are as small as those found in proteins.

Part I[1] describes the volume changes that accompany the reactions of some typical native proteins with hydrogen and hydroxyl ions. It was found that in these proteins the reaction

$$-COO^- + H^+ \longrightarrow -COOH \qquad (1)$$

is accompanied by a volume change of about 11 ml., which (as has been noted by Weber and Nachmansohn[2,3]) is similar to the values observed in

(1) J. Rasper and W. Kauzmann, *J. Am. Chem. Soc.*, **84**, 1771 (1962).

(2) H. H. Weber and D. Nachmansohn, *Biochem. Z.*, **204**, 215 (1929).

(3) H. H. Weber, *ibid.*, **218**, 1 (1930).

simple carboxylic acids. On the other hand, the reaction

$$\begin{matrix}\diagdown\\ -\mathrm{NH}^+\\ \diagup\end{matrix} + \mathrm{OH}^- \longrightarrow \begin{matrix}\diagdown\\ -\mathrm{N}\\ \diagup\end{matrix} + \mathrm{H_2O} \qquad (2)$$

in these proteins brings about a volume change of only 15 to 18 ml., which is 6 to 9 ml. less than is observed in the analogous reactions of simple amines. This is an indication that the environments of the basic groups in proteins differ significantly from those of the simple amines, and it would be interesting to learn the nature of these differences. One approach to this problem is to study the volume changes accompanying reactions 1 and 2 in simple molecules in which the environments of the carboxyl and amino groups are systematically changed. On the basis of such a study one might hope to be able to make inferences about the environments of the charged groups in proteins. This paper reports the results of such a study.

A number of relevant measurements have been reported in the literature. Weber[3] measured the volume changes for the ionization of eight organic acids, nine organic bases and nine amino acids and peptides. He used a dilatometric method, but his precision was limited to about 5%. Linderstrøm-Lang and Jacobsen[4] studied the ionization of ammonia, acetic acid, glycine ethyl ester and alanyl glycyl ethyl ester dilatometrically with a precision similar to that attained in the present work. Other studies[5-8] of volume changes for ionization reactions have been based on differences between the apparent molar volumes of the substances involved in the reaction, the volume being determined usually from solution density measurements over a range of concentrations. In many instances the density determinations were made at concentrations well above 0.1 molar and the extrapolation to dilute solutions introduces a significant uncertainty. (For the reaction $H^+ + OH^- \rightarrow H_2O$ this extrapolation introduces an error of 2.1 ml. in the volume change at infinite dilution.[9]) A summary of volume changes for some simple ionic reactions is given by Harned and Owen.[10]

Experimental

The Carlsberg dilatometers used in this work have been described in Part I.[1] A major limitation of these dilatometers is the requirement that all substances involved in the ionization reaction be insoluble in the kerosene or other fluid layer used to separate the reacting solutions in the dilatometer assembly. This makes it difficult to apply the technique to organic acids and bases which contain large non-polar groups. It is observed, for instance, that after hydrochloric acid is added to sodium trimethylacetate or after sodium hydroxide is added to triethylamine hydrochloride, a continuous, slow increase in volume takes place with time. Agitation greatly increases the rate of this volume change. Evidently the uncharged acid or base produced on mixing the respective solutions is soluble in the kerosene layer, and the transfer from the aqueous layer to the kerosene layer is accompanied by a volume increase. (Such a volume increase is consistent with the observation that non-polar substances generally seem to have much smaller apparent molar volumes in water than they do in hydrocarbon and other non-polar solvents. See, for instance, the measurements by Masterton of the volumes of methane, ethane, propane and benzene in water.[11]) An attempt to circumvent this difficulty by replacing kerosene with a silicone oil was unsuccessful because of differential wetting effects of the water and the oil, which made it impossible to mix the solutions in the two legs of the dilatometer. Mercury would be an ideal fluid for this purpose, but the fact that it is denser than water requires complete redesign of the dilatometer, and we have not been able to devise a way of doing this that preserves the great advantages of simplicity and ease of operation of the Carlsberg design.

The substances studied were used as purchased, without further purification. Their purity was, however, checked by titration. One of the advantages of the dilatometric method as used in the work reported here is its relative insensitivity to impurities. If an impurity does not react with acid or base in the *p*H range employed, it will have no effect on the volume change. On the other hand, if it does react it must be present in relatively large amounts in order to have appreciable effects. The sample of tetra-N-(hydroxyethyl)-ethylenediamine ("Nalco L-652") was donated by the National Aluminate Corp., Chicago, Ill. All measurements were made at 30.0°.

The dilatometer capillaries used here are the same as those used in Part I. Their calibration was checked both by measuring the length of a short column of mercury as it was moved up and down the scale and by weighing a column of mercury equal in length to the full scale. The capillaries were found to be consistent with each other within 0.5% and correct in absolute reading within 1.5%.

Effects of Concentration Changes During an Experiment. —Masson[12] has shown that the apparent molar volumes of most strong electrolytes vary linearly with the square root of the concentration, even in relatively concentrated solutions

$$\phi = \phi_0 + S\sqrt{c} \qquad (3)$$

where ϕ is the apparent molar volume at concentration c and ϕ_0 and S are empirical constants. The constant S for uni-univalent electrolytes at 25° is found to have values between 0.8 and about 4 provided that the concentration range used for fitting data to equation 3 lies above 0.5 to 1 molar. (See Harned and Owen[10,p.361] for a table giving ϕ_0 and S for a large number of electrolytes.) On the other hand, the Debye–Hückel theory predicts a relationship of the same form as Masson's equation in dilute solution,[13] but it predicts that the constant S will have the same numerical value for all electrolytes of a given valence type. According to Redlich and Bigeleisen[7] the Debye–Hückel value of S is 1.86 for uni-univalent electrolytes at 25°, but this value is far from certain because of our imperfect knowledge of the dependence of the dielectric constant of water on the pressure. A survey of the most reliable available data on the concentration dependence of the apparent molar volumes of electrolytes in dilute solution shows that $d\phi/d\sqrt{c}$ approaches a value between 1.8 and 2.0 as c approaches zero.[9]

Thus we have the interesting situation that the same equation is valid in two different ranges of concentration but requires different sets of constants in the two ranges. The transition from one set of constants to the other appears to take place between about 0.1 M and 1 M for most electrolytes.[9] (Hydrochloric acid, however, seems to be exceptional in that the transition takes place below 0.1 M.)

Because of this dependence of ϕ on c, the volume changes that accompany ionic reactions depend appreciably on the concentrations of the electrolytes involved, and this must either be minimized or be taken into account in making comparative studies of volume changes. The magnitude of the effect may be judged by considering the reaction

$$\text{Na acetate} + \text{HCl} \longrightarrow \text{NaCl} + \text{undissociated acetic acid} \qquad (4)$$

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[4] K. Linderstrøm-Lang and C. F. Jacobsen, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **24**, 1 (1941).

[5] J. Daniel and E. J. Cohen, *J. Am. Chem. Soc.*, **58**, 415 (1936).

[6] B. B. Owen and S. R. Brinkley, *Chem. Revs.*, **29**, 461 (1941).

[7] O. Redlich and J. Bigeleisen, *ibid.*, **30**, 171 (1942).

[8] S. D. Hammann and S. C. Lim, *Australian J. Chem.*, **7**, 329 (1954).

[9] A. Bodanszky and W. Kauzmann, *J. Phys. Chem.*, **66**, 177 (1962).

[10] H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," A.C.S. Monograph No. 137, 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1958, p. 405.

[11] W. L. Masterton, *J. Chem. Phys.*, **22**, 1830 (1954).

[12] D. O. Masson, *Phil. Mag.* [7], **8**, 218 (1929).

[13] O. Redlich and L. Rosenfeld, *Z. physik. Chem.*, **A155**, 65 (1931)

The apparent molar volumes at 25° of the reactants and products have been carefully investigated by Redlich and Bigeleisen (hydrochloric acid between 0.0087 and 0.66 *M*,[14] Redlich and Nielsen (sodium acetate and acetic acid[15]) and Wirth (hydrochloric acid between 0.04 and 5.4 *M* and sodium chloride between 0.04 and 4.0 *M*[16]). These measurements show that if reaction 4 is performed at infinite dilution, there would be an expansion of 11.47 ml. at 25°. If, on the other hand, one were to mix equal volumes of 1 *M* sodium acetate and 1 *M* hydrochloric acid to form a solution 0.5 *M* in sodium chloride and 0.5 *M* in undissociated acetic acid, the volume change of reaction 4 would be only 9.60 ml. Mixing equal volumes of 0.1 *M* sodium acetate and 0.1 *M* hydrochloric acid results in a volume change of 10.81 ml., and initial concentrations of 0.05 *M* produce a volume change of 10.99 ml. It is apparent that the electrolyte concentrations can have appreciable effects on the volume changes even in fairly dilute solutions.

Since the main purpose of the present study is to compare the effects of molecular environments of acidic and basic groups on the volume changes, it is desirable to minimize that part of the effect of the concentration changes that varies in going from one electrolyte to another. This can be accomplished by using, wherever possible, concentrations that are sufficiently low that the Debye-Hückel limiting law is obeyed. The different electrolytes that are being compared will then have the same value of the constant *S* in equation 3. It is felt that this condition will be adequately (though by no means precisely) satisfied if the organic acids and bases are used at concentrations below 0.1 *M*. We have been able to meet this condition in most of our experiments. It has, however, been necessary to use higher electrolyte concentrations in experiments with the stronger organic acids, such as chloroacetic acid, in order to reduce the magnitude of the correction for hydrogen ions remaining unbound after addition of hydrochloric acid to the sodium salt of the acid. These corrections are described in the next section.

Corrections for Free Protons and Hydroxyl Ions.—When hydrochloric acid is added to an excess of, say, sodium acetate, most of the protons are bound by the acetate ion to form undissociated acetic acid. A few ions, however, remain free in the solution and produce a change in the *p*H, and in order to calculate the volume change per mole of acetic acid formed, one must divide the observed volume change by the number of moles of hydrogen ions that have actually reacted. If the initial and final *p*H values are greater than about 4, the number of moles reacting is very nearly the same as the number of moles of hydrochloric acid added. If, however, one is studying a stronger acid than acetic acid, it is necessary to make a correction for the unbound hydrogen ions. (A similar correction is necessary in studying the volume changes accompanying the reaction of hydroxyl ions with the protonated forms of very strong bases.) The correction is made by estimating the hydrogen ion concentrations from a comparison of the *p*H of the solution with the *p*H readings obtained on a series of hydrochloric acid solutions of similar, and known, concentrations. (Known sodium hydroxide solutions are, of course, used in the case of the strong bases.)

If the *p*H of the solutions is more than about 3, in the case of the acids, then the correction for free acid or base is considerably reduced by adding the hydrochloric acid in the dilatometer to a mixture of the organic acid with its sodium salt, rather than to the sodium salt alone. The *p*H values before and after adding the hydrochloric acid are then nearly the same, so that there is only a small change in the amount of free hydrogen ion in the solution of the organic salt. A similar situation holds for the addition of sodium hydroxide to the protonated bases when the *p*H is less than about 11. In general we have tried to add hydrochloric acid to a solution containing two parts of organic acid anion and one part of undissociated organic acid, and we have tried to add sodium hydroxide to a solution containing two parts of protonated base to one part of free base. The amount of hydrogen ion or hydroxide ion added is usually sufficient to change these ratios from the initial value of 2:1 to a final value of 2:3. For the strongest acids and bases studied, however, it was felt necessary to keep the free hydrogen or hydroxide ion concentration lower than is possible with this procedure, so only the ionic forms were present initially, and their concentration was also raised so as to keep the final *p*H within a reasonable range for the *p*H meter.

The *p*H values were measured with a Beckman *p*H-meter in some instances and with a Radiometer *p*H-meter in others.

Results

The results of various series of measurements are given in Tables I–IV, each table being subdivided according to the concentration conditions employed. Because of the effect of electrolyte concentrations on the volume changes, the values reported for the different series are not exactly comparable, but in each series at least one reaction was run which was common to some other series, so that an approximate comparison is possible. It is felt that these results are accurate to within about 1% in most instances; where they are less accurate, this is indicated by writing the last digit below the line or by dropping it altogether. Each of the numbers given in the tables is the average of at least two independent dilatometric measurements; in most instances the results of four or more independent runs were averaged in obtaining each reported volume change.

Tables V–VII give volume changes that have been reported by other workers. The agreement with our results is generally satisfactory, considering the differences in electrolyte concentration and temperature employed. Except for the reactions of formate and butyrate ions with the hydrogen ion, the volume changes reported by Hammann and Lim[8] appear to be larger by several milliliters than our results and most of the results of other workers. Perhaps the most significant comparison is with the value for the reaction of acetate ion with hydrogen ion as determined by Redlich and Bigeleisen,[14] who used pycnometric data obtained at relatively high dilution, and whose extrapolations appear to be considerably more reliable than those in other pycnometric measurements. They found that at infinite dilution this reaction is accompanied by a volume change of 11.47 ml. Our measurements for the addition of one volume of 0.2 *N* HCl to 5 volumes of a mixture containing 0.1 *M* sodium acetate and 0.05 *N* acetic acid gave a volume change of 10.9 ml. per mole of protons reacting. Using the data of Redlich and Bigeleisen[14] for the concentration dependence of the apparent molar volume of hydrochloric acid, the data of Redlich and Nielsen[15] for sodium acetate and acetic acid and the data of Wirth[16] for sodium chloride, we find that in going from our concentrations to infinite dilution our value of 10.9 ml. would be increased by 0.63 ml. giving a value of 11.5 ml. for the acetate reaction at infinite dilution, in excellent agreement with Redlich and Bigeleisen's value of 11.47 ml.

The consistency of the data in Table II with those of Table I can be tested in the following way. Since the reaction

$$\text{R-COOH} + \text{NH}_3 \longrightarrow \text{R-COO}^- + \text{NH}_4^+ \qquad (4)$$

is the sum of the reactions

$$\text{R-COOH} \longrightarrow \text{R-COO}^- + \text{H}^+ \qquad (5)$$

and

$$\text{NH}_3 + \text{H}^+ \longrightarrow \text{NH}_4^+ \qquad (6)$$

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[14] O. Redlich and J. Bigeleisen, *J. Am. Chem. Soc.*, **64**, 758 (1942).

[15] O. Redlich and L. E. Nielsen, *ibid.*, **64**, 761 (1942).

[16] H. E. Wirth, *ibid.*, **62**, 1128 (1940).

TABLE I

VOLUME CHANGES FOR THE REACTION $\text{R-COO}^- + \text{H}^+ \rightarrow \text{R-COOH}$

A. Initial solutions: $\begin{cases} \text{1 vol. 0.2 } N \text{ HCl, } xM \text{ NaCl} \\ \text{5 vols. (0.1 } M \text{ NaA, 0.05 } M \text{ HA, } xM \text{ NaCl)} \end{cases}$

Final solution: 6 vols. (0.05 M NaA, 0.075 M HA, (0.033 + x) M NaCl)

Acid formed	ΔV, ml.	
	$x = 0$	$x = 0.15$
Formic	7.8	7.5
Acetic	10.9	10.6
Propionic	12.6	12.3
n-Butyric	14.4	14.2
Isobutyric	14.4	..
Trimethylacetic	~18	..
Phthalic	12.5	..
H·phthalate$^-$	16.5	..
Oxalic	9.5	..
H·oxalate$^-$	16.1	..
Malonic	8.5	..
H·malonate$^-$	18.1	..
Succinic	11.9	..
H·succinate$^-$	12.8	..
H·fumarate$^-$	10.9	..
H·maleate$^-$	22.1	..

B. Initial solutions: $\begin{cases} \text{1 vol. 0.2 } N \text{ HCl} \\ \text{5 vols. 0.2 } M \text{ NaA} \end{cases}$

Final solution: 6 vols. (0.133 M NaA, 0.033 M HA, 0.033 M NaCl)

Acid formed	ΔV, ml.
Acetic	10.9
Oxalic	10.5
H·Oxalate$^-$	15.0
Maleic	7.4
H·maleate$^-$	20.8
Fumaric	9.9
H·fumarate$^-$	10.8
H·malonate$^-$	17.0
H·succinate$^-$	12.4

C. Initial solutions: $\begin{cases} \text{1 vol. 0.2 } N \text{ HCl} \\ \text{5 vols. 0.4 } M \text{ NaA} \end{cases}$

Final solution: 6 vols. (0.3 M NaA, 0.033 M HA)

Acid formed	ΔV, ml.
Acetic	9.7
Acrylic	10.8
Methacrylic	12.7
Glycolic	9.5
Thioglycolic	10.1
Lactic	11.7
Pyruvic	12
Chloroacetic	9.2
Dichloroacetic	11
Bromoacetic	9.4
Cyanoacetic	7.2

D. Initial solutions: $\begin{cases} \text{1 vol. 0.2 } N \text{ HCl} \\ \text{5 vols. 0.1 } M \text{ amino acid} \end{cases}$

Final solution: 6 vols. (0.05 M amino acid, 0.033 M amino acid·HCl)

Amino acid	ΔV, ml.
Glycine	6.4
α-Alanine	7.6
β-Alanine	7.5
Phenylalanine	8.3

TABLE II

VOLUME CHANGES FOR THE REACTION[a]

$$\text{R-COOH} + \text{NH}_3 \longrightarrow \text{R-COO}^- + \text{NH}_4{}^+$$

Initial solutions: $\begin{cases} \text{1 vol. (0.05 } M \text{ NH}_4\text{Cl, 0.05 } M \text{ NH}_3\text{)} \\ \text{2 vols. (0.05 } M \text{ NaA, 0.05 } N \text{ HA)} \end{cases}$

Final solution: 3 vols (0.017 M NH_4A, 0.017 N HA, 0.017 M NH_4Cl, 0.033 M NaA)

Acid, R-COOH	ΔV, ml.	δ_A, ml.[b]	δ_B, ml.[c]
Acetic	−17.5	−6.6	−6.6
(H fumarate)$^-$	−17.0	−6.1	−6.2
(H maleate)$^-$	−28.2	−6.1	−7.4
(H oxalate)$^-$	−22.1	−6.0	−7.1
(H malonate)$^-$	−24.3	−6.2	−7.3
(H succinate)$^-$	−18.9	−6.0	−6.5
(H adipate)$^-$	−19.0	...	...
(H_3 citrate)^0	−14.9	...	...
(H_2 citrate)$^-$	−18.8	...	...
(H citrate)$^=$	−24.4	...	...
(H isopropylene malonate)$^-$	−25.0	...	...

[a] These measurements and those given in Table III were made during a visit to the Carlsberg Laboratory, Copenhagen, by one of us (W. K.), who wishes to express his appreciation for the opportunity to use the facilities of this Laboratory, provided by its Director, Prof. K. Linderstrøm-Lang. [b] Sum of ΔV given in this Table and the value given for the corresponding acid in Table IA. [c] Sum of ΔV given in this Table and the value given for the corresponding acid in Table IB.

TABLE III

EFFECT OF CONCENTRATION OF SODIUM CHLORIDE ON THE VOLUME CHANGE OF THE REACTION

$$(\text{H maleate})^- + \text{NH}_3 \longrightarrow \text{maleate}^= + \text{NH}_4{}^+$$

Initial solutions: $\begin{cases} \text{1 vol. (0.01 } M \text{ NH}_4\text{Cl, 0.01 } M \text{ NH}_3\text{, } xM \text{ NaCl)} \\ \text{1 vol. (0.0133 } M \text{ Na}_2 \text{ maleate, 0.0133 } M \text{ NaH maleate, } xM \text{ NaCl)} \end{cases}$

Final solution: 2 vols. (0.0117 M maleate$^=$, 0.0017 M H maleate$^-$, 0.01 M $NH_4{}^+$, (x +0.02)M Na^+, xM Cl^-)

x, molarity of NaCl	0	0.2	1.0	4.1
ΔV, ml.	27.8	25.7	22.3	19.3

TABLE IV

VOLUME CHANGES FOR THE REACTION

$$\text{R}_1\text{R}_2\text{R}_3\text{NH}^+ + \text{OH}^- \longrightarrow \text{R}_1\text{R}_2\text{R}_3\text{N} + \text{H}_2\text{O}$$

A. Initial solution: $\begin{cases} \text{1 vol. (0.2 } N \text{ NaOH, } xM \text{ NaCl)} \\ \text{5 vols. (0.10 } M \text{ amine·HCl, 0.05 } M \text{ amine, } xM \text{ NaCl)} \end{cases}$

Final solution: 6 vols. (0.05 M amine·HCl, 0.075 M amine, (x + 0.017)M NaCl)

Base formed	ΔV, ml.	
	$x = 0$	$x = 0.15$
Ammonia	27.0	26.4
Methylamine	24.9	24.3
Ethyl amine	24.6	..
n-Propylamine	25.1	..
Dimethylamine	24.4	24.0
Diethylamine	24.4	..
Trimethylamine	26.0	..
Hydroxylamine	24.7	..
Hydrazine	26.7	..
Triethanolamine	..	25.1
Ethylenediamine	26.0	..
Ethylenediamine·H^+	31.5	..
Tetra-N-(hydroxyethyl)-ethylenediamine	21.7	..
Tetra-N-(hydroxyethyl)-ethylenediamine·H^+	35.6	..
Na_3 ethylenediaminetetraacetate (*p*H 9)	22.6	..

B. Initial solutions: $\left\{\begin{array}{l}\text{1 vol. 0.2 } N \text{ NaOH} \\ \text{5 vols. (0.1 } M \text{ amino acid or amine·HCl)}\end{array}\right.$

Final solution: 6 vols. (0.05 M amino acid or amine·HCl), 0.033 M Na salt of amino acid, or amine

Base formed	ΔV, ml.
Ammonia	25.0
Ethylenediamine	27.3[a]
Ethylenediamine·H$^+$	30.5[a]
Na salt of glycine	22.1
Na salt of α-alanine	21.2
Na salt of β-alanine	22.6
Na salt of phenylalanine	21.2

C. Initial solutions: equal volumes of 0.05 M amine·H$^+$ and varying concentrations of NaOH (between 0 and 0.05 N)

Amino acid or base	ΔV, ml.
Histidine	
Imidazole, *p*H 5–7	23.1
α-amino, *p*H 8–10	21
Imidazole	22.4
Histidine methyl ester	
Imidazole, *p*H 7.5–8.5	24.5
Acetylhistidine	
Imidazole, *p*H 7–8	19.8
Histamine	
Imidazole, *p*H 4–6	25
Amino, *p*H >6	24.2
Arginine	
α-amino, *p*H 8–10	21.7
Lysine	
ϵ-amino, *p*H 10–11	22

[a] 0.2 M amine salt used.

TABLE V

VOLUME CHANGES FOR REACTIONS OF HYDROGEN IONS WITH CARBOXYLATE GROUPS

Method A, from apparent molar volumes of reactants and products, determined from solution densities; B, measured in dilatometer.

Acid formed	ΔV, ml.	Method	Ref.
Formic	8.0	A	(1)
Acetic	9.2	A	(2)
	11.47	A	(3)
	12.5	A	(1)
	10.3	B	(4)
Propionic	13.7	A	(1)
	12.6	B	(4)
n-Butyric	13.7	A	(1)
Lactic	11.4	B	(4)
Carbonic	29	A	(2)
Bicarbonate ion	27.8	A	(2)
Oxalic	7.2	B	(4)
H·oxalate$^-$	15	B	(4)
Glycine·H$^+$	5.9	A	(5)
	7.1	B	(4)
Alanine·H$^+$	8.1	B	(4)
Proline·H$^+$	6.9	B	(4)
Oxyproline·H$^+$	7.4	B	(4)
Histidine·H$^+$	7.7	B	(4)
β-Aminobutyric·H$^+$	7.4	B	(4)
ϵ-Amino caproic·H$^+$	13.2	A	(4)
Acetyl glycine	11.7	B	(4)
Glycylglycine·H$^+$	9.2	B	(4)
Alanyl glycine·H$^+$	10.2	B	(4)
Leucylglycine·H$^+$	10.6	B	(4)
Alanylglycylglycine·H$^+$	10.1	B	(6)

[1] Hammann and Lim.[8] This work was done at 25°, apparent molar volumes being measured between 0.1 and 1 M and extrapolated to infinite dilution. [2] Owen and Brinkley.[6] Based on density measurements at 25° and concentrations from about 0.3 to 1 or 2 M. [3] Redlich and Bigeleisen.[7] Densities and apparent molar volumes were measured to unusually high dilutions, in the range 0.01 to 0.8 M at 25°. [4] Weber.[3] Concentrations of 0.1 M were employed for the organic electrolyte in most instances, but for stronger organic acids and bases mixtures of the free acid or base with its salt at a total concentration of 0.2 to 0.3 M was employed. The HCl and NaOH concentrations were $N/15$ to $N/5$. The temperature was 30°. [5] Daniel and Cohen.[5] Apparent molar volumes were determined at concentrations usually in the range between 0.2 to 0.3 M and 1.5 to 4 M. These values were then extrapolated to zero concentration. The temperature was 25°. [6] Linderstrøm-Lang and Jacobsen.[4] Electrolyte concentrations were 0.05 to 0.1 M and the temperature was 20°. [7] Weber and Nachmansohn.[2] Conditions as in ref. 4, above. [8] Bodanszky and Kauzmann.[6] $t = 30°$.

TABLE VI

VOLUME CHANGES FOR REACTIONS OF HYDROXYL IONS WITH PROTONATED AMINO GROUPS

Base formed	ΔV, ml.	Method[a]	Ref.[a]
Ammonia	30.0	A	(1)
Methylamine	27.5	A	(1)
	27.0	B	(4)
Dimethylamine	27.4	A	(1)
	24.3	B	(4)
Trimethylamine	28.1	A	(1)
Ethylamine	24.5	B	(4)
Piperidine	24.3	A	(1)
Aniline	28.6	A	(1)
	25.6	B	(4)
Pyridine	28.1	A	(1)
	27.0	B	(4)
Imidazole	23.9	B	(4)
Guanidine	23.2	B	(4)
Glycinate$^-$	21.8	A	(5)
	23.6	B	(4)
Alaninate$^-$	22.0	B	(4)
Prolinate$^-$	20.9	B	(4)
Oxyprolinate$^-$	20.7	B	(4)
Histidinate$^-$ (*p*H 4–7)	23.6	B	(4)
β-Aminobutyrate$^-$	22.5	B	(4)
ϵ-Aminocaproate$^-$	24.3	A	(5)
Creatinate$^-$	20.4	B	(4)
Glycine ethyl ester	26.1	B	(4)
Glycylglycinate$^-$	25.7	B	(4)
Alanylglycinate$^-$	25.3	B	(4)
Leucylglycinate$^-$	25.5	B	(4)

[a] See Table V.

TABLE VII

VOLUME CHANGES ACCOMPANYING MISCELLANEOUS IONIC REACTIONS

Reaction	ΔV, ml.	Method[a]	Ref.[a]
$H_3O^+ + OH^- \rightarrow 2H_2O$	23.4	A	(2)
	21	B	(7)
	21.3	A and B	(8)
$H_3O^+ + NH_3 \rightarrow H_2O + NH_4^+$	− 6.8	B	(6)
NH_3 + acetic acid $\rightarrow NH_4^+$ + acetate$^-$	−17.4	B	(6)
(Same reaction in 1 N KCl)	−15.4	B	(6)
H_3O^+ + glycine ethyl ester $\rightarrow$	− 5.6	B	(6)
glycine ethyl ester·H$^+$ + H_2O	− 5.8	B	(4)
H_3O^+ + Na alanylglycylglycinate $\rightarrow$ alanylglycylglycine + H_2O	− 5.1	B	(6)
H_3O^+ + Phenolate$^-$ $\rightarrow$	17.0	A	(1)
phenol + H_2O	18.7	B	(4)

[a] See Table V.

the volume change of reaction 6 is the difference of the volume changes of reactions 4 and 5. This difference is shown in Table II as δ_A and δ_B, δ_A being the sum of ΔV for a given acid in Table II and ΔV for the same acid in Table IA, and δ_B being the sum of ΔV for a given acid in Table II and ΔV for the same acid in Table IB. The values of δ_A and δ_B agree well with the value of −6.8 ml. obtained for reaction 6 by Linderstrøm-Lang and Jacobsen[4] (see Table VII). The variations in δ_A and δ_B for the different acids undoubtedly are caused by the different electrolyte concentrations employed in Tables IA, IB and II. The variations, it should be noted, are in the right direction if the electrolyte concentration effect on apparent molar volumes is the greatest for those multicharged ions whose charges are closest together (*i.e.*, oxalate, maleate and malonate as compared with fumarate and succinate). The existence of such an effect will be discussed below.

Discussion

Effects of Substituents on the Volume Changes. —Hammann and Lim[8] have pointed out that the volume change accompanying the ionization of a carboxylic acid, R-COOH, appears to depend quite strongly on the nature of the alkyl group that is attached to the carboxyl, whereas the volume change of the reaction between hydroxyl ion and protonated bases, $R_1R_2R_3NH^+$, depends to a much lesser extent on the number and nature of the alkyl groups attached to the nitrogen atom. This observation is supported by our results, especially those in which the groups R_1, R_2 and R_3 are uncharged. Thus the volume change for the carboxyl ionization reaction varies from −7.2 ml. in cyanoacetic acid and −7.8 ml. in formic acid to more than −14 ml. in *n*-butyric acid and trimethylacetic acid, whereas for amino compounds the volume changes for the reaction of hydroxyl with the protonated base vary only from 27.0 ml. for ammonia to 24.4 ml. for dimethylamine and diethylamine. This observation is quite remarkable. The ionic charge of the ammonium ion is presumably equally distributed among the four hydrogen atoms that are bonded to the nitrogen atom; one would expect that the replacement of these charged protons by alkyl groups would have a considerable effect on the arrangement of water molecules around the ion and therefore on the apparent volume occupied by the ion. In the carboxylic acids the charge is located on oxygen atoms, and the substitutions are made at some distance from the charge—either on the adjacent carbon atom (in going from formic acid to acetic acid) or on the carbon atom once further removed (in going from acetic acid to propionic, isobutyric and trimethylacetic acids). A smaller effect of substituents on the volume would therefore have been anticipated in the acids than in the amines.

Even more striking is the greatly different effect of introducing a hydroxyl group onto a carboxyl and an amino group, as shown by the reactions

$\text{H-COOH} \longrightarrow \text{H-COO}^- + \text{H}^+$ $\quad \Delta V = -7.8$ ml.
$\text{HO-COOH} \longrightarrow \text{HO-COO}^- + \text{H}^+$ $\quad \Delta V = -29$ ml.[10]
Diff. = 21 ml.

$\text{H-NH}_3^+ \longrightarrow \text{H-NH}_2 + \text{H}^+$ $\quad \Delta V = -6.8$ ml.[4]
$\text{HO-NH}_3^+ \longrightarrow \text{HO-NH}_2 + \text{H}^+$ $\quad \Delta V = -3.7$ ml.[17a]
Diff. = 3.1 ml.

A similar but less striking contrast is seen on comparing the effect of introducing an oppositely charged group near the ionizing group

$\text{H-CH}_2\text{-COOH} \rightarrow \text{H-CH}_2\text{COO}^- + \text{H}^+$ $\quad \Delta V = -10.9$ ml.
$\text{NH}_3^+\text{-CH}_2\text{-COOH} \rightarrow \text{NH}_3^+\text{-CH}_2\text{-COO}^- + \text{H}^+$ $\quad \Delta V = -6.4$ ml.
Diff. = − 4.5 ml.

$\text{H-CH}_2\text{-NH}_3^+ \rightarrow \text{H-CH}_2\text{-NH}_2 + \text{H}^+$ $\quad \Delta V = -3.9$ ml.
$^-\text{OOC-CH}_2\text{-NH}_3^+ \rightarrow {}^-\text{OOC-CH}_2\text{-NH}_2 + \text{H}^+$ $\quad \Delta V = -1.1$ ml.
Diff. = − 2.8 ml.

$\text{H-CH}_2\text{-CH}_2\text{-COOH} \rightarrow \text{H-CH}_2\text{-CH}_2\text{-COO}^- + \text{H}^+$ $\quad \Delta V = -12.6$ ml.
$\text{NH}_3^+\text{-CH}_2\text{-CH}_2\text{-COOH} \rightarrow \text{NH}_3^+\text{-CH}_2\text{-CH}_2\text{-COO}^- + \text{H}^+$ $\quad \Delta V = -7.5$ ml.
Diff. = − 5.1 ml.

$\text{H-CH}_2\text{-CH}_2\text{-NH}_3^+ \rightarrow \text{H-CH}_2\text{-CH}_2\text{-NH}_2 + \text{H}^+$ $\quad \Delta V = -3.6$ ml.
$^-\text{OOC-CH}_2\text{-CH}_2\text{-NH}_3^+ \rightarrow {}^-\text{OOC-CH}_2\text{-CH}_2\text{-NH}_2 + \text{H}^+$ $\quad \Delta V = -1.6$ ml.
Diff. = − 2.0 ml.

$\text{H-CH(CH}_3\text{)-COOH} \rightarrow \text{H-CH(CH}_3\text{)-COO}^- + \text{H}^+$ $\quad \Delta V = -12.6$ ml.
$\text{NH}_3^+\text{-CH(CH}_3\text{)-COOH} \rightarrow \text{NH}_3^+\text{-CH(CH}_3\text{)-COO}^- + \text{H}^+$ $\quad \Delta V = -7.6$ ml.
Diff. = − 5.0 ml.

$\text{H-CH(CH}_3\text{)-NH}_3^+ \rightarrow \text{H-CH(CH}_3\text{)-NH}_2 + \text{H}^+$ $\quad \Delta V = -3.6$ ml.
$^-\text{OOC-CH(CH}_3\text{)-NH}_3^+ \rightarrow {}^-\text{OOC-CH(CH}_3\text{)-NH}_2 + \text{H}^+$ $\quad \Delta V = -0.2$ ml.
Diff. = − 3.4 ml.

These results show that the effect of the introduction of a charged amino group close to a carboxyl group on the ΔV of dissociation of the carboxyl group is about twice as large as the effect of introducing a carboxylate group on the ΔV of dissociation of an NH_3^+ group. Further discussion will be given below of the effects of charges in the vicinity of a group on the ΔV of dissociation of the group.

The volume changes accompanying the two stages of dissociation of tetra-N-(hydroxyethyl)-ethylenediamine are distinctly different from those of the other amines. Thus the removal of a proton from the singly charged ion with hydroxyl ion gives a volume change of only 21.7 ml., which is less than that observed for any other singly charged amino compound studied by us or reported elsewhere. Furthermore, the removal of a proton from the doubly charged ion produces a volume change of 35.6 ml., which is the largest value observed for any amino compound.

The volume change for the reaction of the imidazolium group with hydroxyl ion appears to lie in the range of 20 to 25 ml., which is about the same as the values observed for amino compounds (see Table IVC). Hammann and Lim[8] have reported that for pyridine and aniline the corresponding

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(17) (a) This value is obtained by combining the result from hydroxylamine in Table IV with the volume change of −21.0 for the reaction $\text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^-$ as measured dilatometrically by Weber[3] and by W. Kauzmann, unpublished measurements.

reactions produce volume changes of 28.1 ml. and 28.6 ml., respectively, and Weber[3] found a value of 25.6 ml. for the aniline reaction. Since Hammann and Lim's values appear to be generally larger than ours and other workers' by several milliliters, it would appear that these aromatic bases also give volume changes quite close to those of aliphatic amines. Thus we may conclude that the reaction of hydroxyl ions with the protonated forms of all nitrogenous bases is accompanied by a volume change of 20 to 25 ml., regardless of whether the base is an aliphatic amine, an aromatic amine or a heterocyclic compound with the basic nitrogen atom in the ring. Volume changes outside this range are found only when additional charged groups are present in the molecule.

Drude and Nernst[18] showed that if a charge Z_e is uniformly distributed on the surface of a sphere of radius a and immersed in a dielectric, the dielectric will undergo a volume change (electrostriction)

$$\Delta V = -\beta(V\mathrm{d}D/\mathrm{d}V)e^2Z^2/2aD^2 \qquad (7)$$

where β, D and V are respectively the compressibility, dielectric constant and volume of the dielectric. From the point of view of this simple model one might expect that the substitution of alkyl groups into the formate and ammonium ions would increase the effective sizes of the ions and would therefore decrease the electrostriction of the surrounding solvent. Although a small decrease in volume does seem to accompany the introduction of alkyl groups into the ammonium ion, the opposite effect is observed for the carboxylate ion. Thus the factors that determine the apparent volumes of these ions in water solution must be more complex than is envisioned in the Drude–Nernst model. It is probable that the most important influence is the effect of the alkyl groups (and other groups as well) on the structure of the water in the vicinity of the ion, and the study of the volumes of these ions would appear to be an especially good means of learning about this structure.

Effect of Net Charge on the Volume Change.—The Drude–Nernst model predicts that the electrostriction of the solvent around an ion is proportional to the square of the charge on the ion. Consider the reactions

$$H^+ + A^{n-} \longrightarrow HA^{(n-1)-}$$

$$H^+ + HA^{(n-1)-} \longrightarrow H_2A^{(n-2)-}$$

$$\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots$$

$$H^+ + H_mA^{(n-m)-} \longrightarrow H_{m+1}A^{(n-m-1)-}$$

$$\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots$$

$$H^+ + H_{n-1}A^- \longrightarrow H_nA$$

If the hydrogen ion (or more properly the hydronium ion, H_3O^+) and the anions $H_mA^{(n-m)-}$ are assumed to be spheres of radii a_H and a_m, then according to the Drude–Nernst theory (equation 7) the volume change accompanying the reaction of a proton with $H_mA^{(n-m)-}$ should be

$$\Delta V = -K\left[\frac{(n-m-1)^2}{a_{m+1}} - \frac{(n-m)^2}{a_m} - \frac{1}{a_H}\right]$$

where K is the coefficient $\beta e^2(V\mathrm{d}D/\mathrm{d}V)/2D^2$ in equation 7, a positive quantity depending only on the properties of the solvent. Let us assume that all of the anions have the same radius, $a_m = a\ (0 \leqslant m \leqslant n)$. Then

$$\Delta V_m = K\left[\frac{2(n-m)-1}{a} + \frac{1}{a_H}\right] \qquad (8)$$

For a dibasic acid, such as oxalic acid, $n = 2$, and the reaction of a proton with the doubly charged anion $A^{=}$ gives a volume change

$$\Delta V_0 = K\left[\frac{3}{a} + \frac{1}{a_H}\right]$$

whereas the reaction with the singly charged anion HA^- gives

$$\Delta V_1 = K\left[\frac{1}{a} + \frac{1}{a_H}\right] = \Delta V_0 - 2\frac{K}{a}$$

Thus the reaction of H^+ with $A^{=}$ should give a larger volume change than the reaction of H^+ with HA^-, by an amount equal to double the electrostriction of HA^-. Our measurements show that for those dibasic acids in which the two carboxyl groups are close together (as in oxalic acid, malonic acid, phthalic acid and maleic acid), the doubly charged anion does give a considerably larger volume change than is found with an ordinary carboxyl group—the difference ranging as high as 13.4 ml. for maleic acid. On the other hand, if the carboxylate groups in a doubly charged dibasic acid anion are sufficiently far apart that their electrostatic fields do not overlap appreciably, the electrostriction of each will be independent of the state of ionization of the other. This is the situation in fumaric, succinic and adipic acids. In fumaric acid the two carboxyl groups occupy *trans*-positions relative to a carbon-carbon double bond and the distance between them is much greater than in, say, maleic acid, where these groups are in a *cis*-relationship. In the doubly charged succinate anion freedom of rotation about the carbon-carbon single bond between the two methylene groups that join the carboxyl groups, together with the mutual coulombic repulsion that exists between the carboxylate groups when both are in the ionic form, will keep the carboxylate groups well separated from each other. In the adipate anion, where the carboxylate groups are joined by four methylene groups, the separation can be even greater. This is why the volume change in the second stage of the ionization of fumaric, succinic and adipic acids is not very different from that in the first stage of ionization (Table IA, B).

A comparison of the volume changes in the successive ionization steps of citric acid and succinic acid is interesting in this connection. From the data presented in Table II we find that the volume changes in the binding of a proton by the ions H_2Cit^-, $HCit^{=}$ and $Cit^{\equiv}$ are 8.8 ml., 12.7 ml. and 18.3 ml., respectively. In citric acid three carboxyl groups are attached at the 1, 2 and 3 positions of a three carbon atom chain, so each group is separated from the others by at least two carbon atoms. In succinic acid, where the carboxyl groups are also separated by two carbon atoms, the volume changes in the binding of a proton by $HSuc^-$ and $Suc^{=}$ are 11.9 ml. and 12.8 ml.,

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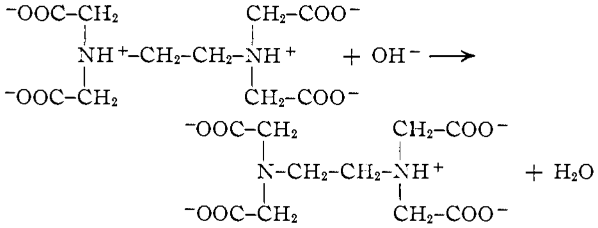
(18) P. Drude and W. Nernst, *Z. physik. Chem.*, **15**, 80 (1894).

respectively, a difference of only 0.9 ml. (see Table IA). In citric acid the corresponding difference is four times greater (12.7 − 8.8 = 3.9 ml.). These results probably indicate that the interaction of the carboxylate ions in $HCit^{=}$ is considerably greater than in $Suc^{=}$, and in the triply charged $Cit^{\equiv}$ ion the interaction must be even greater, in spite of the considerable distance between the groups. (The volume changes in the citric acid ionizations more or less parallel the free energy changes of these reactions. After correcting for the Bjerrum statistical factor the ΔF^0 values for the first, second and third ionizations are 4920 cal., 6490 cal. and 8060 cal., respectively, at 25°.[19] The increment in ΔF^0 here is 1570 cal. between each successive step. In the two ionization steps of succinic acid the ΔF^0 values (again corrected for the Bjerrum statistical factor) are 6160 cal. and 7270 cal. at 25°.[19] The increment here is 1110 cal., which is not very much smaller than the increment for the citric acid ionizations. Thus the free energy changes do not indicate as great a difference in the carboxyl group interactions in citric acid and succinic acid as do the dilatometric measurements.)

Thus we find that if two negatively charged groups are close together in an anion, so that their electrostatic fields overlap appreciably, the total electrostriction in the anion $A^{=}$ is more than double that in the singly charged anion HA^{-}. It is convenient to refer to this as the *reinforcing effect* on the electrostriction.

That similar reinforcing effects occur in cations of the type H_2C^{++} is evident from the volume changes that accompany the reactions of hydroxyl ions with the doubly charged dibasic cations from ethylenediamine and from tetra-N-(hydroxyethyl)-ethylenediamine (see Table IV). It is, however, interesting that the reinforcing effect is much greater in tetra-N-(hydroxyethyl)-ethylenediamine than in ethylenediamine itself. In the former compound the difference between ΔV for removal of the first and second protons from the ion is 14 ml. whereas in ethylenediamine this difference is about 3.5 to 5.5 ml. It is also interesting to note that in both of these compounds the two amino groups are separated by two carbon atoms, so that the charge separation is not very different from that in succinic acid. Yet in succinic acid the difference in ΔV in the first and second ionization is only about one milliliter—much less than is observed here.

Measurements were made of the volume change accompanying the reaction of hydroxyl ion with one of the two dissociable protons of the disodium salt of ethylenediaminetetracetic acid (Na_2EDTA). In these experiments the mixture of Na_2EDTA and Na_3EDTA in the dilatometer had a *p*H of 8.1 before addition of the alkali and 9.0 after the addition, so the reaction that takes place is undoubtedly The observed volume change was 22.6 ml. (Table IV). This is to be compared with the volume changes of more than 30 ml. obtained with the analogous reactions forming ethylenediamine·H^+ and tetra-N-(hydroxyethyl)-ethylenediamine·H^+,



$$(^{-}OOC\text{-}CH_2)_2NH^{+}\text{-}CH_2\text{-}CH_2\text{-}NH^{+}(CH_2\text{-}COO^{-})_2 + OH^{-} \longrightarrow (^{-}OOC\text{-}CH_2)_2N\text{-}CH_2\text{-}CH_2\text{-}NH^{+}(CH_2\text{-}COO^{-})_2 + H_2O$$

and the volume changes of 21 to 23 ml. for the reaction of hydroxide ion with α-amino acids. Evidently the reinforcing effect of two adjacent positively charged amino groups is more than compensated by the four negatively charged carboxylate ions.

These qualitative predictions of the Drude–Nernst theory are also substantiated by comparing the volume changes of the amino acids with those of the parent acids and bases, such as

$$H^+ + NH_3^+\text{-}R\text{-}COO^- \longrightarrow NH_3^+\text{-}R\text{-}COOH \qquad (9a)$$

as compared with

$$H^+ + R\text{-}COO^- \longrightarrow R\text{-}COOH \qquad (9b)$$

and

$$OH^- + {}^{-}OOC\text{-}R\text{-}NH_3^+ \longrightarrow {}^{-}OOC\text{-}R\text{-}NH_2 + H_2O \qquad (10a)$$

as compared with

$$OH^- + R\text{-}NH_3^+ \longrightarrow R\text{-}NH_2 + H_2O \qquad (10b)$$

In reactions 9a and 10a the charged species H^+ or OH^- reacts with the over-all neutral species $NH_3^+\text{-}R\text{-}COO^-$ to form a charged species (whose radius will be assumed to be a), so that (*cf.* equations 7 and 8)

$$\Delta V_a = K\left[\frac{1}{a_0} - \frac{1}{a}\right]$$

where a_0 is the radius of the hydronium ion or the hydroxyl ion. In reactions 9b and 10b H^+ or OH^- reacts with a charged species (assume its radius is a') giving the volume change

$$\Delta V_b = K\left[\frac{1}{a_0} + \frac{1}{a'}\right]$$

It is clear that in each of the pair of reactions 9 or 10 ΔV_b should be larger than ΔV_a. This is observed on comparing the reactions of glycine with those of methylamine and acetic acid, and the reactions of α- and β-alanine with those of ethylamine and propionic acid (Table VIII). It is interesting to note, however, that despite the fact that the charged groups in isoelectric β-alanine are separated by two carbon atoms, as compared with α-alanine, where they are separated by only one carbon atom, nevertheless the differences between ΔV_a and ΔV_b for β-alanine are about the same as the differences

TABLE VIII

COMPARISON OF THE VOLUME CHANGES OBSERVED FOR REACTIONS 9 AND 10

	ΔV (ml.) for reaction			
Parent amino acid	(9a)	(9b)	(10a)	(10b)
Glycine	6.4	10.9	22.1	24.9
α-Alanine	7.6	12.6	21.2	24.6
β-Alanine	7.5	12.6	22.6	24.6
ε-Aminocaproic acid	13.2	(14)[a]	24.3	(25)[a]

[a] Values estimated from volume changes for other compounds of similar structure.

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[19] R. G. Bates and G. D. Pinching, *J. Am. Chem. Soc.*, **71**, 1274 (1949); *J. Res. Natl. Bur. Stand. U. S.*, **45**, 322, 444 (1950).

for α-alanine. This suggests that an intramolecular salt linkage must exist between the NH_3^+ group and the COO^- group in aqueous solutions of β-alanine. It is also interesting that in ϵ-aminocaproic acid, where there are five carbon atoms between the amino and carboxyl groups, the volume change for reaction 9a is very close to that expected for (9b), and the volume change for (10a) is very close to that expected for (10b). It would therefore appear that an intramolecular salt linkage does not exist in ϵ-aminocaproic acid.

These results demonstrate that if two carboxyl groups are close together in a molecule (that is, at distances similar to those in oxalic, maleic, malonic and phthalic acids), we may expect that the volume change of the reaction of the doubly charged anion with a hydrogen ion will be abnormally large by 5 to 10 ml. or more. There is a similar effect in the reaction of protonated amino groups with hydroxyl ion if two such groups are close together. On the other hand, if a charged amino group is close to a carboxylate ion (separations similar to those in α and β amino acids), then the volume change accompanying the reaction of the carboxyl group with a proton is reduced by about 5 ml. and the volume change accompanying the reaction of the protonated amino group with hydroxyl ion is reduced by 2 to 3 ml.

It should be noted that in the polyamines and polycarboxylic acids such as polyvinylamine and polyacrylic acid these reinforcing effects of adjacent charged groups also seem to take place.[20]

Effects of Added Electrolyte and Urea on the Volume Changes.—The studies reported in Part I on the volume changes accompanying the reactions of proteins with hydrogen ions and hydroxyl ions were performed in 0.15 *M* sodium chloride whereas most of the present studies were made in the absence of added electrolyte. The effect of 0.15 *M* sodium chloride was therefore studied for a few of the reactions involving singly charged ions. The results are shown in Tables I and IV. It is found that this concentration of sodium chloride decreases ΔV by only a few tenths of a milliliter. Such a decrease is expected because the apparent molar volumes of nearly all ionic substances increase as the electrolyte concentration is increased and this decreases the magnitude of the difference in volume between the neutral and ionized forms of the organic acids and bases. Thus the effect of 0.15 *M* sodium chloride is much too small to affect the interpretation of the measurements on the protein.

Higher concentrations of electrolyte have much greater effects, however. Linderstrøm-Lang and Jacobsen[4] have reported that for the reaction of acetic acid with ammonia to form acetate ion and ammonium ion the volume change is altered from -17.4 ml. in water to -15.4 ml. in 1 *M* KCl. Table III shows there is an even larger effect of sodium chloride on the volume change accompanying the reaction of the hydrogen maleate ion with ammonia to form the doubly charged maleate ion and ammonium ion. As already has been noted, volume changes involving maleate ion are unusually large because of a reinforcing effect of the carboxylate ions on each other's electrostriction. This reinforcement is evidently much reduced by high concentrations of sodium chloride. As has been noted already, the nature of the slight deviation from constancy in the last column in Table II is another indication of the sensitivity of the volume occupied by doubly charged anions to added electrolyte.

The effect of urea on the volume change of the acetic acid–ammonia reaction also has been studied. When a solution containing 0.1 *M* sodium acetate and 0.1 *N* acetic acid was added to an equal volume of a solution containing 0.1 *M* ammonium chloride and 0.1 *N* ammonia, the volume change was -16.8 ml. if the solvent was water and -15.0 ml. if the solvent was 7 *M* urea. Thus strong urea somewhat reduces the volume change of the reaction, though its effect is smaller on a mole-for-mole basis than that of simple electrolytes.

Relationship between Volume Changes and Changes in Other Thermodynamic Properties.—It is interesting to compare the trends in the volume changes accompanying the ionic dissociation reactions of a series of carboxylic acids and amines with the changes in other thermodynamic properties of the same reactions. Fig. 1 shows that rough correlations may indeed exist between the values of ΔV and the values of ΔF, ΔH and ΔS in the ionization of the carboxylic acids. The introduction of a substituent which makes ΔV more negative tends to make ΔF more positive and ΔH and ΔS more negative in these compounds. On the other hand the relationship between ΔV and ΔC_p for the carboxylic acids seems to be more complex; in the homologous series from formic acid to *n*-butyric acid ΔC_p becomes less negative as ΔV becomes more negative, but for the polar amino acids and dibasic acids ΔC_p becomes more negative as ΔV becomes more negative.

For the hydrolysis of the amines there is, as already has been pointed out, a much smaller change in ΔV when different substituents are introduced, and Fig. 1 shows that there is almost no correlation at all between these small changes in ΔV and the rather large changes that occur in the other thermodynamic quantities.

In this connection it is significant that there do seem to be definite correlations between ΔS and ΔH and between ΔS and ΔC_p for the ionization reactions of both the carboxylic acids and the amines. Figure 2 shows that for both classes of compounds ΔH tends to become more positive as ΔS becomes more negative; furthermore, the variations for the acids and the amines seem to fall more or less along a single slightly curved line. The relationship between ΔS and ΔC_p is less striking, but it would appear that as ΔS becomes more negative, ΔC_p becomes more negative for the acids and less negative for the amines.

The question of the existence of correlations between ΔV, ΔS and ΔC_p in these reactions is important for the understanding of the environments of ionizing groups. In contrast to ΔH and ΔF, these three properties of the ionization reaction

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[20] J. Rasper, A. Bodanszky and W. Kauzmann, to be published.

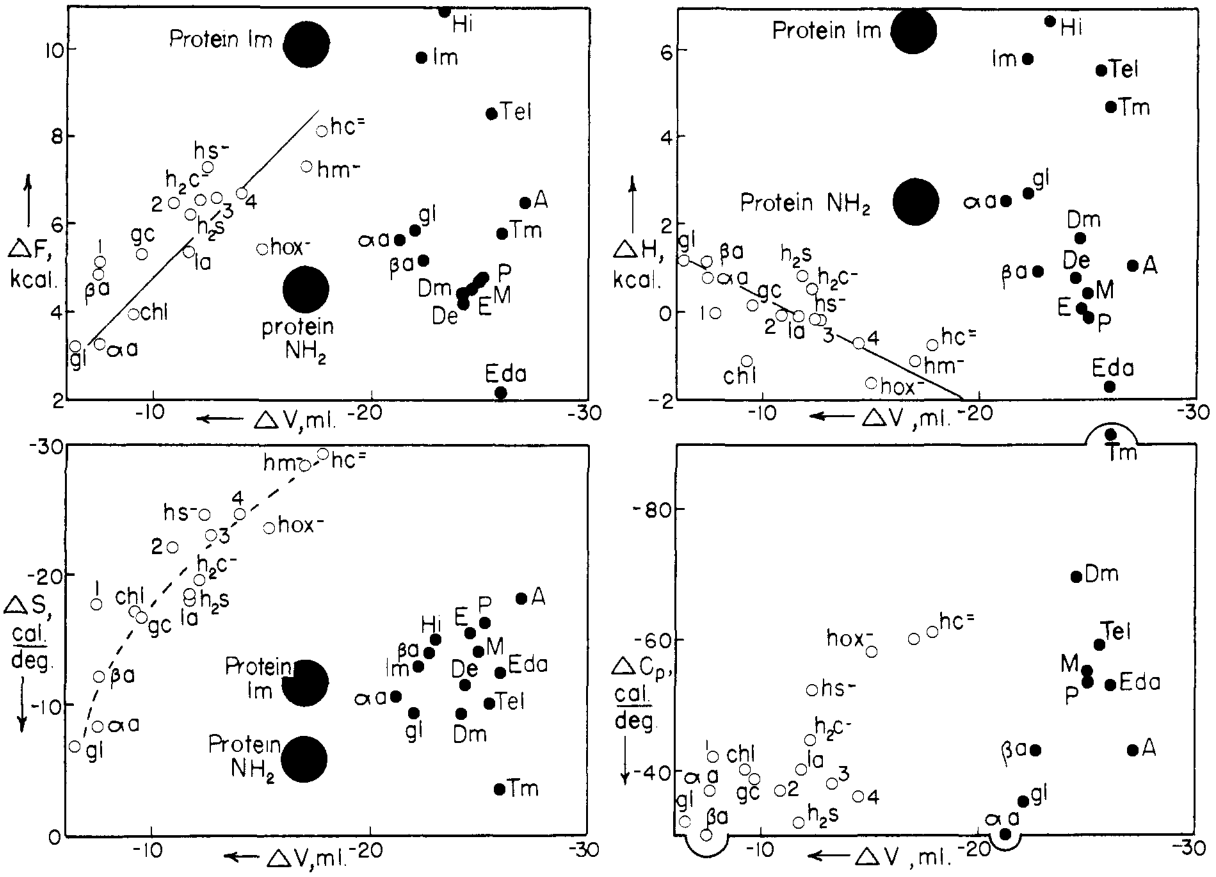

Fig. 1.—Relationships between the value of ΔV and the values of ΔF, ΔH, ΔS and ΔC_p for the ionic dissociation reactions of carboxylic acids and amines. Open circles, carboxylic acids ($R—COOH \rightarrow R—COC^- + H^+$); filled circles, amines ($R_1R_2R_3N + H_2O \rightarrow R_1R_2R_3NH^+ + OH^-$). Symbols identify the substances corresponding to individual points in Figs. 1 and 2 as follows; *Acids:* 1, formic; 2, acetic; 3, propionic; 4, butyric; gc, glycolic; la, lactic; chl, chloroacetic; h_2s, succinic; hs^-, H. succinate$^-$; hox^-, H. oxalate$^-$; hm^-, H. malonate$^-$; gl, glycine; αa, α-alanine; βa, β-alanine; h_3c, h_2c^- and $hc^=$, three stages of ionization of citric acid; αb, α-aminobutyric. *Amino compounds:* A, ammonia; M, methylamine; Dm, dimethylamine; Tm, trimethylamine; E, ethylamine; De, diethylamine; Te, triethylamine; P, *n*-propylamine; B, *n*-butylamine; El, ethanolamine; Del, diethanolamine; Tel, triethanolamine; Eda, ethylenediamine; Hda, hexamethylenediamine; Hi, histidine (imidazole): Im, imidazole. Data on ΔF, ΔH, ΔS and ΔC_p are from the tabulations of Harned and Owen,[10] Bates and Hetzer,[21] Bates and Pinching[19] and Edsall and Wyman.[23] The general locations of the imidazole and ε-amino groups of proteins are indicated by the large filled circles.

undoubtedly are affected mainly by the structure of the solvent around the molecules involved in the reactions, especially for those reactants and products that bear electric charges. (Although ΔH and ΔF are affected by changes in the solvation accompanying the ionization reactions, they are also greatly influenced by changes that take place within the molecule that is undergoing ionization, particularly by changes in bond energies, which may be considerable because of the intramolecular electronic induction and resonance effects that result when different substituents are present. These electronic shifts would not be expected to have as great an influence on ΔV, ΔS and ΔC_p.) Let us suppose that the solvent around a charged solute ion such as $R\text{-}COO^-$ can exist in only two extreme structures and that as the substituent R is altered only the relative amounts of these two structures are changed. Then ΔS, ΔC_p and ΔV would each be related in some simple way to the relative amounts of the two structures. If this were the case a strict correlation would exist between the three properties, and a plot of any one of them against either of the other two would give a smooth curve—in fact, a straight line. Since a simple and strict correlation of this kind does not seem to be observed, a more complex situation must exist in the hydration layers around these ions. Evidently the apparent molar volume emphasizes different aspects of the structure of the hydration layers around the ions than do the enthalpy, entropy and heat capacity.

In looking for correlations between volume changes and energy changes of ionization reactions, it is helpful to have some notion of the heat and free energy that one must supply in order to change the volume in the hydration layer around a solute. What is the energy required to bring about a volume change of, say, one milliliter per mole of solute in these systems? There are, of course, many ways of changing volumes and each

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(21) R. G. Bates and H. B. Hetzer, *J. Phys. Chem.* **65**, 671 (1961).

method will require a different energy, but we should like to know the general orders of magnitude that are involved in order to gain some feeling for the energy changes that might be associated with a given change in ΔV.

It is well known that formation of a hole having the size of a molecule in a liquid requires an energy equal to the heat of vaporization. Thus the introduction into water of 18 ml. of molecular voids would require the introduction of 10,500 cal. at 20°, or 585 cal./ml. of space created. Another method of changing the volume of water is by heating, and the cost in energy per unit volume is given by the ratio of the specific heat to the coefficient of thermal expansion. For water this ratio varies between 5,000 cal./ml. at 20° to 1,700 cal./ml. at 70°. Apparently simple heating is an expensive method of creating space in water, presumably because most of the heat supplied goes into modes of motion that have little or no effect on the volume. A third method of changing the volume of a liquid is to immerse a rigid conducting sphere of radius a in the liquid and cover it with a charge q. The free energy change in the charging process is $q^2/2Da$, where D is the dielectric constant of the liquid. The volume change accompanying the charging process is (*cf.* eq. 7) $-q^2(\mathrm{d}D/\mathrm{d}p)/2aD^2$ where $\mathrm{d}D/\mathrm{d}p$ is the change in dielectric constant with pressure. The ratio of the free energy change to the volume change is $-D(\mathrm{d}p/\mathrm{d}D)$. For water this ratio is about $-20{,}000$ atm., or -490 cal./ml.[22] A fourth method of changing the volume of water is by direct isothermal compression. The free energy required to compress one mole of water by one milliliter at 25° is 1,100 cal. If n moles of water are compressed by one milliliter, the energy required is $1{,}100/n$ cal. Suppose that each mole of a solute is hydrated by n moles of water and a substituent is introduced into the solute which has the effect of compressing hydrostatically the hydration water in its vicinity to the extent of one milliliter. The free energy required will be $1{,}100/n$ cal. If n = 5 to 10, the compression requires 100 to 200 cal./ml., and if only a portion of the hydration layer is compressed, a correspondingly larger energy per unit volume change will be required.

Where correlations exist between the volume changes and the free energy and enthalpy changes, so that straight lines may be drawn on the plots of ΔF *vs.* ΔV and ΔH *vs.* ΔV in Fig. 1, the slopes of these lines are found to be about -500 cal./ml. for $\mathrm{d}\Delta F/\mathrm{d}\Delta V$ and 300 cal./ml. for $\mathrm{d}\Delta H/\mathrm{d}\Delta V$. The observed value of -500 cal./ml. for $\mathrm{d}\Delta F/\mathrm{d}\Delta V$ agrees remarkably well with the value of -490 cal./ml. calculated above for the process of charging a sphere in water.

The above considerations indicate that a change in ΔV amounting to several milliliters brought about by some change in the environment of a carboxyl group or an amino group may well be accompanied by changes in ΔF and ΔH amounting to one or more kilocalories and might be reflected in changes in the dissociation constant by one or more powers of ten.

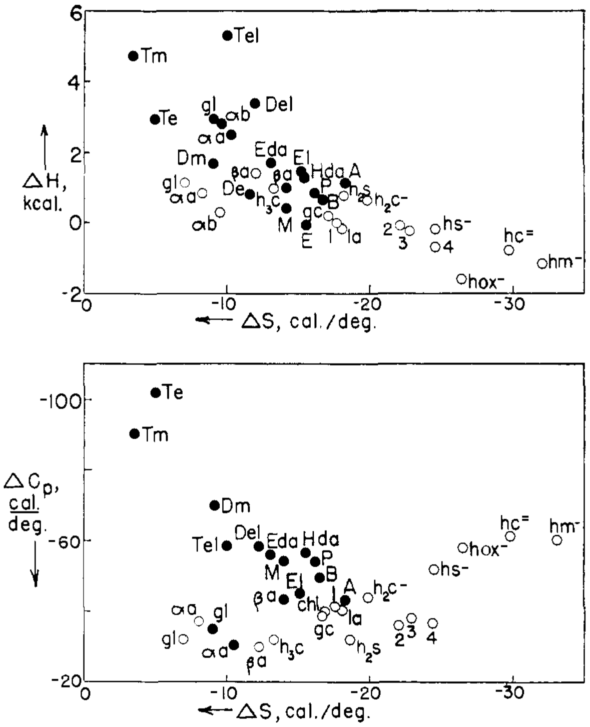

Fig. 2.—Relationships between the value of ΔS and the values of ΔH and ΔC_p for the ionic dissociation reactions of carboxylic acids and amines. Symbols and sources of data are identified in Fig. 1.

Significance of the Volume Changes Observed in the Reactions of Proteins with Hydrogen and Hydroxyl Ions.—In Part I we have confirmed the earlier observations of Weber and Nachmansohn that ΔV for reaction 1 in proteins has a value of 10 to 12 ml., which is well within the range of values observed for simple carboxylic acids. This justifies the widely held belief that the great majority of the side chain carboxyl groups of the common globular proteins behave normally in their dissociation reactions. On the other hand, it was found that ΔV for reaction 2 in proteins is only 15 to 18 ml., and this is less by 5 to 10 ml. than the values of 22 to 25 ml. observed for a wide variety of simple molecules containing amino and imidazole groups. This result is surprising in view of the fact that the volume changes in reaction 2 in simple molecules seem to be quite insensitive to such drastic changes in the environment as the replacement of hydrogen atoms on the amino group by alkyl groups and the introduction of negatively charged groups in their immediate vicinity. Evidently the environments of the amino and imidazole groups of proteins contain features that are not duplicated in the model compounds listed in Tables IV and VI. It is even more remarkable that these unusual features seem to have little or no effect on the free energies and enthalpies of dissociation of these groups in proteins. Thus it is generally believed that both the titration curves of globular proteins at room temperature and the displacements of these curves brought about by temperature changes are readily accounted for if both the intrinsic dissociation constants of the imidazole and ε-amino

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(22) O. Redlich, *J. Phys. Chem.*, **44**, 619 (1940); F. E. Harris, E. W. Haycock and B. J. Alder, *ibid.*, **57**, 978 (1953).

groups in proteins and the temperature coefficients of these dissociation constants are given the same values as those observed in simple compounds containing the same groups (see Edsall and Wyman[23]). Yet we have seen in the last section that a change of 5 to 10 ml. from the normal value of ΔV might well be accompanied by changes of three or more kilocalories in ΔF, which would bring about a change in the dissociation constant of at least two powers of ten. There is, of course, no indication that such changes actually occur.

We are unable to offer an explanation for the anomalous behavior of these volume changes in proteins. In view of the small effect of the carboxylate ion in α- and β-amino acids on the ΔV of the amino group, it hardly seems likely that carboxylate ions near the protein amino groups can account for the anomaly. That hydrophobic regions in the vicinity of the amino groups could in some way decrease the volume changes seems unlikely because of the small effects on ΔV resulting from the substitution of alkyl groups directly onto the amino group, as in mono-, di- and trimethylamines and ethylamine. If the amino groups were embedded to any appreciable extent in a non-aqueous environment of low dielectric constant, the electrostriction would be much reduced because $V(\mathrm{d}D/\mathrm{d}V)$ in equation 7 is much smaller for such an environment than for water. It is, however, difficult to believe that this could be the case here, because such a situation would surely be accompanied by a large increase in the acidity of the protonated amino groups, which is not observed. It is conceivable that the ionization of the basic groups of proteins is accompanied by a conformation change that would bring about the anomalous volume change that we have observed. One would then expect that other properties of proteins that are sensitive to conformation (intrinsic viscosity, optical rotation) would change in the pH range in which the amino groups titrate. There is no evidence that this is the case, but it is conceivable that the conformation changes may be involved here which are of a type that do not affect, say, the optical rotation. We are inclined to suspect that this is the basic reason for the anomaly, but it is difficult to see why such conformation changes are not accompanied by free energy changes that would be reflected in anomalous dissociation constants of the basic groups of proteins. The layers of frozen water postulated by Klotz[24] may also be responsible for the anomaly, but aside from the objections that have been raised to this hypothesis,[25] it is not evident to us how these layers could bring about the observed behavior. Furthermore, Klotz has used his hypothesis in order to explain the anomalous reactivities of specific groups, such as sulfhydryl groups, and the groups we are concerned with here are not believed to have an anomalous reactivity.

Studies also have been made recently of the volume changes that accompany the reaction of wool and hair with acids and bases.[26] The studies show that in these substances reaction 1 is accompanied by a volume change of 9 ml., and reaction 2 is accompanied by a volume change of only 11 ml. Thus the carboxyl groups of wool and hair appear to react normally, but the basic groups deviate to an even greater extent from their behavior of simple molecules than do the basic groups in soluble proteins.

Acknowledgments.—This work was supported by a grant from the National Science Foundation.

(23) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1958, pp. 525–526.

(24) I. M. Klotz, *Science*, **128**, 815 (1958); *Brookhaven Symposia in Biology*, **13**, 25 (1960).

(25) W. Kauzmann, *Advances in Protein Chem.*, **14**, 1 (1959); *Brookhaven Symposia in Biology*, **13**, 43 (1960).

(26) D. D. Kasarda and W. Kauzmann, to be published.

[CONTRIBUTION FROM THE PHYSICAL CHEMISTRY DIVISION, NATIONAL CHEMICAL LABORATORY, POONA-8, INDIA]

A Study of the Interaction of Nickel(II) with Bovine Serum Albumin[1a]

BY M. S. NARASINGA RAO

RECEIVED NOVEMBER 21, 1961

The binding of Ni^{II} by bovine serum albumin at pH 6.5 (acetate buffer of ionic strength 0.20) has been measured by the pM indicator method, using murexide as the indicator, and by the equilibrium dialysis technique. There is good agreement in the values obtained by the two methods. The electrophoretic mobility of albumin has been measured as a function of the bound Ni^{II}. The binding data supplemented by the mobility data lead to a value of $\log k^0 = 3.17$ for the intrinsic association constant. This compares well with the first association constant, $\log k = 3.27$, for Ni^{II}–imidazole interaction, suggesting that the Ni^{II} is bound at the imidazole groups of the albumin molecule. The values of $\log k^0$ decrease with $\bar{\nu}$. This has been attributed to the increased participation of carboxyl groups in the interaction.

Introduction

The interaction of Cu^{II}, Zn^{II}, Cd^{II} and Co^{II} with bovine serum albumin has been studied and the mode of interaction appears to differ among the metals.[1b] It was of interest, therefore, to study nickel–albumin interaction and to compare it with the other systems.

Recently, Ettori and Scoggan[2] have measured the binding of calcium ions by albumin with the pM indicator, murexide. Quantitative binding data have been obtained by this and the equilibrium dialysis technique to examine the applicability of this method to nickel–albumin interaction.

Experimental

Crystallized bovine serum albumin was an Armour product lot No. CB0271. It was deionized by passing an

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(1) (a) Communication No. 481 from National Chemical Laboratory, Poona-8 (India). (b) M. S. Narasinga Rao and Hira Lal, *J. Am. Chem. Soc.*, **80**, 3226 (1958).

(2) J. Ettori and S. M. Scoggan, *Arch. Biochem. Biophys.*, **91**, 27 (1960).