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The Location of Electrostatic Charges in Kirkwood's Model of Organic Ions

BY CHARLES TANFORD[1]

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The interaction between electrostatic charges on organic ions was treated by Kirkwood in 1934 in terms of a model which formally represents the organic ion as a cavity of low dielectric constant in a solvent continuum. This paper shows that a crucial parameter in determining interaction energies on the basis of this model is the depth, *d*, within the cavity, at which charges or dipoles are placed. To account for experimentally observed interaction energies, as reflected in the dissociation constants of appropriate organic acids (Kirkwood–Westheimer theory), it is necessary that *d* be assigned a value close to 1.0 Å. for discrete charges and about 1.5 Å. for dipoles. This result is in agreement with that found earlier in an extension of the Kirkwood model to charge interactions on protein molecules. An empirical procedure, based on this finding, permits the direct calculation of interaction energies, and, hence, of *pK* differences between related acids differing by the presence of charged or dipolar substituents.

The properties of organic molecules in solution which depend on the interaction between electrostatic charges upon them have been treated with considerable success by the model proposed by Kirkwood[2] in 1934. In this model the organic ion is treated as a cavity of low dielectric constant (ordinarily placed equal to 2), embedded in the solvent, which is treated as a continuum with its macroscopic dielectric constant. Using this model, one can show[3] that the free energy of interaction between a pair of charges, q_1 and q_2, separated by a distance R, can be expressed as

$$W = \frac{q_1 q_2}{D_E R} \tag{1}$$

where D_E is a parameter with the dimensions of a dielectric constant, the value for which depends primarily on the location of the charges with respect to the interface between the cavity and the solvent. Values of D_E for spherical and ellipsoidal cavities have been given in tabular and graphical form in a number of places.[3–5]

A similar equation may be written[3] for the interaction between a charge q and a point dipole of moment μ

$$W = \frac{q\mu \cos \zeta}{D_E R^2} \tag{2}$$

Here R is the distance between the charge and the dipole, and ζ the angle between the dipole axis and the line joining the charge to the dipole. Values of D_E for use in eq. 2 have been tabulated by Westheimer and co-workers.[3,4]

The principal application of the Kirkwood model has been in the procedure used by Kirkwood and Westheimer[3] to account quantitatively for differences in the dissociation constants of related acids on the basis of electrostatic interaction between the dissociating proton and a charge or dipole on another part of the molecule. The present calculations result from an attempt to extend this kind of calculation to proteins. It becomes immediately apparent that such a calculation will depend not only on an estimation of the size of a protein molecule and on the distance between charged sites, both of which one could hope to obtain with fair accuracy from a suitable model, but that there is also a *critical* dependence on the depth of the charges below the surface of the cavity which represents the molecule. This is illustrated, for example, by the calculations of Table I. These show that the depth of charges (d) is a considerably more important variable than the size of the molecule.

Clearly, if the Kirkwood model is to be extended to proteins, an assumption must be made concerning this crucial parameter. Since in many proteins most of the charged sites appear to be at the molecular surface, in direct contact with the solvent, it is reasonable to assume that in these cases the protein charges will tend to be about as close to the cavity surface as they are in small organic ions. Accordingly the calculations below were made to obtain typical values of d in such ions.

Calculations Based on Differences in Acid Dissociation Constants

The Kirkwood–Westheimer theory[3] relates the difference between acid dissociation constants of certain related acids to the interaction between a single pair of charges, or between a charge and a point dipole. The depth of charges below the cavity surface does not enter explicitly into the calculation. Instead, suitable assumptions are made concerning the shape and structure of the organic molecule, which in effect express all other variables, including the depth d, in terms of the distance R between the interacting charges. This distance is then computed from observed ratios of dissociation constants.

The values of R so obtained from the Kirkwood–Westheimer treatment are nearly always reasonable values. Since, for a given value of R, the interaction energy depends so critically on the depth d, this means that the assumed structure parameters must have been well chosen, and therefore suitable for a calculation of d. Thus, for the case where ΔpK depends on the interaction between a pair of charges on a sphere, Table I shows that a "wrong" value of d would lead to a "wrong" value of D_E (by as much as a factor of three for an error of 1 Å.), which, by eq. 1, could be compensated for only by a correspondingly "wrong" value of R, W being the experimental quantity obtained from the pK difference.

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(1) Department of Chemistry, State University of Iowa, Iowa City, Iowa. John Simon Guggenheim Memorial Fellow, Yale University, 1956–1957. This work also was supported by research grant RG-2350 from the National Institutes of Health, Public Health Service.

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

(3) J. G. Kirkwood and F. H. Westheimer, *ibid.*, **6**, 506, 513 (1938).

(4) F. H. Westheimer, W. A. Jones and R. A. Lad, *ibid.*, **10**, 478 (1942).

(5) T. L. Hill, *J. Phys. Chem.*, **60**, 253 (1956); THIS JOURNAL, **78**, 5527 (1956).

TABLE I

EFFECTIVE DIELECTRIC CONSTANT[a] FOR INTERACTION OF TWO CHARGES ON A SPHERE,[b] AT A SEPARATION OF 5 Å.

Depth, d, Å.	Radius of sphere, b, Å. 4	5	6	20	40
0	95	86	78	54	47
0.5	51	50	49	36	32
1.0	24	24	24	20	19
1.5	15	15	15	11.5	11.5
2.0	..	10	10	8.5	8.5

[a] Based on an internal dielectric constant of 2, and a solvent dielectric constant of 78. [b] For ellipsoidal cavities D_E is tabulated in the literature only for charges located at the foci of a prolate ellipsoid. For $d << R$ such ellipsoids are elongated and of *much* smaller volume than the smallest sphere compatible with the chosen values of d and R. The result is a much larger value of D_E (for R = 5 Å.) when $d \leqslant 1.0$ Å. For d = 1.5 or 2.0 Å. D_E for an ellipsoid approaches that for a sphere with the same values of d and R.

Accordingly, in Table II, we have made calculations of d for representative dicarboxylic acids listed in extensive tabulations by Westheimer and Shookhoff[6] and by Peek and Hill.[7] Because of symmetry we can assume that in these acids both charged sites occupy equivalent positions. The values of d were obtained by simple trigonometry from the structural parameters assumed by these authors, together with the inter-charge distances calculated by them from observed pK differences. The table clearly shows relatively little variation in d. According to Westheimer and Shookhoff,[6] the choice of somewhat different structural parameters for some of the acids would lead to variation of the order of 0.3 Å. in the calculated value of R. This would affect the calculation of d to the extent of about 0.2 Å. An additional source of error is discussed in footnote 10. Within a reasonable probable error inherent in the calculation, the charge depth in most of the examples of the table is clearly constant, and approximately equal to 1.0 Å.

TABLE II

DICARBOXYLIC ACIDS

Acid	ΔpK^b	D_E	R, Å.	d, Å.
Oxalic	2.36	27	3.85	0.80
Malonic	2.26	26	4.10	.95
Methylmalonic	1.89	32	4.05	.75
Ethylmalonic	2.05	29	4.10	.90
Dimethylmalonic	2.29	26	4.15	.85
Diethylmalonic	*4.48*	*14*	*3.75*	*2.20*
Methylethylmalonic	2.95	20	4.10	1.05
Glutaric	0.47	74	7.00	1.10
β-Methylglutaric	.56	64	6.85	1.15
β-Ethylglutaric	.44	73	7.60	1.20
β-Propylglutaric	.47	67	7.75	1.25
β,β-Dimethylglutaric	*1.97*	*24*	*5.25*	*1.75*
β,β-Diethylglutaric	*3.66*	*14*	*4.70*	*1.70*
β,β-Dipropylglutaric	*3.02*	*14*	*5.15*	*1.70*
Adipic	0.38	83	7.75	1.25
Pimelic	.34	87	8.30	1.05
Suberic	.28	94	9.30	1.05
Azelaic	.26	96	9.85	1.00
Azelaic[a]	.31	86	9.15	1.10
Sebacic[a]	.30	86	9.60	1.15
Dodecanedioic[a]	.26	86	11.20	1.05
Tetradecanedioic[a]	.21	85	13.85	0.85

[a] In 20% methanol as solvent. [b] Corrected for the statistical factor.

TABLE III

pK_1 AND pK_E FOR MALONIC AND GLUTARIC ACIDS[a]

Acid	pK_1	$pK_E - pK_1$	d, Å.
Malonic	2.83	0.56	0.95
Methylmalonic	3.05	.34	.75
Ethylmalonic	2.99	.51	.90
Dimethylmalonic	3.17	.40	.85
Methylethylmalonic	2.86	..	1.05
Diethylmalonic	*2.21*	*1.51*	*2.20*
Glutaric	4.34		1.10
β-Methylgutaric	4.25		1.15
β-Ethylglutaric	4.29		1.20
β-Propylglutaric	4.31		1.25
β,β-Dimethylglutaric	*3.70*		*1.75*
β,β-Diethylglutaric	*3.62*		*1.70*
β,β-Dipropylglutaric	*3.69*		*1.70*

[a] Dissociation constants were taken from ref. 9.

The four substances which are listed in Table II *in italics* are conspicuous exceptions to the generalization just made: in these acids d is closer to 2 Å. than to 1 Å. These same substances, however, show other abnormalities indicating that non-coulombic interaction plays an important role in determining dissociation constants. Thus, if pK differences result entirely from coulombic interaction, pK_1 should be roughly the same for all glutaric acids: pK_1 should differ from the pK of *n*-valeric acid by a statistical factor (log 2) and by the effect of the dipolar COOH group (≃0.2). Similarly, all the malonic acids should have roughly the same pK_1, with somewhat greater variation due to the larger contribution (≃1.7) from the dipolar COOH group. Table III shows the exceptional behavior of the italicized acids of Table II in this regard. Another source of information is the ratio of K_1 to the dissociation constant K_E of the half-ester, ROOC— . . . —COOH. Here the dipolar substituents are essentially identical and, if coulombic interaction is the only kind influencing pK values, K_1 should differ from K_E only by a statistical factor of 2, *i.e.*, $pK_E - pK$ should be equal to 0.30. Measurements of K_E are available only for the malonic acids: again diethylmalonic acid shows a large deviation from the experimental result. It can therefore be concluded that the four italicized acids of Table II are influenced strongly by non-coulombic interactions (*e.g.*, hydrogen bonding[8,9]) so that the calculation of any parameters on the basis of the Kirkwood–Westheimer relations *alone* is meaningless.[10]

Calculation of the charge depth for a number of diamines are reported in Table IV. Here again symmetry requires that both charged sites be placed an equal distance from the cavity surface. It

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[6] F. H. Westheimer and M. W. Shookhoff, THIS JOURNAL, **61**, 555 (1939).

[7] H. M. Peek and T. L. Hill, *ibid.*, **73**, 5304 (1951).

[8] F. H. Westheimer and O. T. Benfey, *ibid.*, **78**, 5309 (1956).

[9] H. C. Brown, D. H. McDaniel and O. Häfliger, in Braude and Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955.

[10] Even some of the acids with d approximately 1.0 Å. do not have $pK_E - pK_1$ exactly equal to 0.3. Therefore the ΔpK values for these and perhaps for most dicarboxylic acids cannot be determined *entirely* by coulombic forces. Some of the variation in d observed in Table II could be due to differences in the adequacy of a purely electrostatic model.

is seen that *d* again varies little from one diamine to another. Again *d* is about 1 Å. or a little larger.

TABLE IV

DIAMINES[a]

	ΔpK[b]	D_E	*R*, Å.	*d*, Å.
1,2-Diaminoethane	2.41	23	4.45	1.40
1,3-Diaminopropane	1.46	34	4.95	1.30
1,4-Diaminobutane	0.93	45	5.85	1.25
1,5-Diaminopentane	.71	54	6.45	1.25
1,8-Diaminoöctane	.30	88	9.25	1.10

[a] Data of Schwarzenbach, as reported in ref. 9. [b] Corrected for the statistical factor.

Table V shows similar calculations based on the dissociation constants of amino acids $^-OOC-\ldots-NH_3^+$, as compared with their esters, $ROOC-\ldots-NH_3^+$. Here it is not necessarily true that *d* is the same for both charges. However, the calculations of Westheimer and Shookhoff,[6] on which the present analysis is based, assume symmetrical positions of the charges. This would be expected to lead to an effective value of *d* intermediate between the values of *d* for the carboxylate and amino charges. Again values of *d* near 1 Å. are obtained, indicating that the charges in these molecules are similarly placed with respect to the cavity surface as are the corresponding charges in symmetrical molecules.

TABLE V

AMINO ACIDS

	ΔpK	D_E	*R*, Å.	*d*, Å.
Glycine	2.02	30	4.05	0.80
Alanine	2.07	31	3.85	.70
β-Alanine	1.06	45	5.15	1.20
γ-Aminobutyric acid	0.72	56	6.10	1.15
δ-Aminovaleric acid	.62	60	6.55	1.20
ε-Aminocaproic acid	.38	82	7.85	1.10
Glycylglycine	.56	67	6.50	1.10

In Table VI these calculations are extended to dipolar substituents. The dipole and charge are again assumed symmetrically placed (at the foci of an ellipsoid) so that the values of *d* listed are again effective depths, intermediate between those appropriate, respectively, to the dipole and charge. The results suggest that dipoles of the type –C–X, if taken to be point dipoles, must be placed at a somewhat greater distance from the cavity surface than discrete charges.

TABLE VI

HALOGENATED CARBOXYLIC ACIDS[a]

Acid	ΔpK	D_E	*R*, Å.	*d*, Å.
Chloroacetic	1.89	3.0	3.10	1.35
Bromoacetic	1.87	3.0	3.10	1.40
Iodoacetic	1.59	3.0	3.20	1.40
β-Chloropropionic	0.85	5.1	4.50	1.40
β-Bromopropionic	.88	4.9	4.45	1.50
β-Iodopropionic	.79	4.7	4.50	1.50
γ-Chlorobutyric	.30	8.0	5.30	1.40
γ-Bromobutyric	.23	8.7	5.60	1.35

[a] The values of *R* in this table are recalculated. Two of the values computed by Westheimer and Shookhoff (ref. 6) appear to be in error.

Intrinsic Dissociation Constants

The discussion so far has been confined to calculations based on *differences* between *pK*'s of related molecules caused by interaction between charges on different sites. However, the intrinsic values of these constants must be subject also to the influence of electrostatic forces. Thus the reaction $-NH_3^+ \rightarrow -NH_2 + H^+$ involves the transfer of a charge from a large cavity of low dielectric constant to a smaller one. The work involved in this transfer, while never explicitly calculated from the Kirkwood theory in the past, can be computed without difficulty. It is found, like interaction energies, to be very sensitive to the choice of *d*. Thus the remarkable constancy of *pK* for the homologous series $CH_3NH_3^+$ to $CH_3(CH_2)_9NH_3^+$ (*cf.* Brown, *et al.*[9]) indicates that *d* must be assigned close to the same value for each member of the series. In fact, that one is able to think of the effect of substituents on acidity generally in terms of *interactions alone*, without having recourse to changes in intrinsic dissociation constants, is itself evidence for little variation in *d* in a series of similar acids.

Direct Calculation of ΔpK

Attempts which have been made in the past to use the Kirkwood–Westheimer theory for the direct calculation of ΔpK have not been conspicuously successful. The usual procedure has been to fix the volume and shape of the cavity and to assign an appropriate value to *R* and to related geometrical factors. The calculation, however, is very sensitive to these choices, so that the calculated ΔpK may vary over a wide range. For glycine, for instance, with an assigned volume obtained by Traube's rule, a variation in *R* from 3.5 to 4.0 Å. leads to ΔpK values ranging from 2.0 to 5.0. If, in addition, the cavity volume is allowed to vary, an even wider range of ΔpK values becomes possible.

The calculations presented in this paper suggest at once why this method of computing ΔpK has not succeeded. For, with volume and shape fixed, the parameter *d* becomes highly sensitive to the remaining geometric factors and to *R*. The relative con-

TABLE VII

DIRECT CALCULATION OF ΔpK FOR AMINO ACIDS AND DIAMINES[a]

	R, Å.	D_E	ΔpK Calcd.	ΔpK Obsd.[b]
1,2-Diaminoethane	3.38	26	2.78	2.41
1,3-Diaminopropane	4.32	41	1.38	1.46
1,4-Diaminobutane	5.26	54.5	0.85	0.93
1,5-Diaminopentane	6.12	68	.59	.71
1,8-Diaminoöctane	8.76	91	.31	.30
Glycine	3.76	32	2.03	2.02
Alanine	3.76	32	2.03	2.07
β-Alanine	4.84	48	1.06	1.06
γ-Aminobutyric acid	5.59	60	0.73	0.72
δ-Aminovaleric acid	6.54	74	.51	.62
ε-Aminocaproic acid	7.30	80	.42	.38
Glycylglycine	6.41	72	.47	.56

[a] The depth *d* was fixed at 1.0 Å. for all calculations. *R* was chosen as half-way between the inter-charge distances for free rotation and maximum extension (*cf.* ref. 6). An ellipsoidal model with charges at the foci was employed. Assigning values to *d* and *R* automatically fixes the volume of the ellipsoid. In general this volume will differ considerably from that which would be calculated by Traube's rule. [b] Corrected for the statistical factor in the case of the diamines.

stancy of the empirical *d* values presented in Tables II, IV, V and VI, and the insensitivity of D_E to the volume (Table I) suggests that better results may be obtained if *d* is kept fixed, rather than the volume. That this is indeed so is shown strikingly by the data of Table VII. Equally good agreement is obtained for straight chain unsubstituted dicarboxylic acids, beginning with succinic acid. In all of these calculations *d* has been fixed at 1.0 Å. and an ellipsoid model with charges at the foci has been used.

When substituted dicarboxylic acids are considered the question arises whether to use a spherical or ellipsoidal model. Empirically it appears (*cf.* Table VIII) that a spherical model yields best agreement with experiment when the approach of solvent to the acidic site is sterically hindered over a substantial solid angle. For the same reason, perhaps, a spherical model works best for malonic acid. On the other hand, when the substituent, such as a single β-substituent on a glutaric acid, interferes but little with the approach of solvent, then an ellipsoidal model appears to give best agreement with experiment.

TABLE VIII

DIRECT CALCULATION OF Δ*pK* FOR DICARBOXYLIC ACIDS[a]

		Δ*pK*		
Acid	*R*, Å.	Calcd. Ellipsoid	Calcd. Sphere	Obsd.[b]
Malonic	4.50	1.27	2.22	2.26
Methylmalonic	4.50	1.27	2.22	1.89
Ethylmalonic	4.50	1.27	2.31	2.05
Glutaric	6.37	0.54	1.05	0.47
β-Methylglutaric	6.37	.54	1.05	.56
β-Ethylglutaric	6.37	.54	1.05	.44

[a] Depth *d* fixed at 1 A.; *R* chosen as half-way between the intercharge distances for free rotation and maximum extension. When using the spherical model, the volume calculated by Traube's rule was used for ethylmalonic acid, the angle θ (ref. 6) then being automatically determined. For all the other acids the Traube volume is too small to allow use of the assigned values of *d* and *R*. For these the minimum volume compatible with *d* and *R* was used. When the ellipsoidal model is used, the volume is fixed by the assigned values of *d* and *R*. [b] Corrected for the statistical factor.

In any event the data for dicarboxylic acids should not be regarded too seriously. It was shown in Table III that non-electrostatic forces play a large role in determining the dissociation constants of four of the acids listed. The same forces can be expected to enter to some extent into the properties of all such acids.[10] The difference in Δ*pK* for malonic and methylmalonic acid (Table VIII) should also be noted. In any purely electrostatic theory Δ*pK* for methylmalonic acid can never be less than that for malonic acid, but this is the observed result.

The direct calculation of Δ*pK* for dipolar substituents strictly requires that the dipole be at a greater depth than the charged site. If currently available expressions for D_E are to be used, however, the dipole and charge must be symmetrically placed. Table VI suggests that an effective depth of about 1.5 Å. might give results in agreement with experiment. This is indeed found to be so. Using Westheimer and Shookhoff's estimates[6] of the angle ζ we can predict reasonably well the Δ*pK* values of the halogenated carboxylic acids. In accord with the discussion on dicarboxylic acids, we get best results using a spherical model for the halogenated acetic acids and an ellipsoidal model for the propionic and butyric acids.

Table IX shows similar calculations applied to two substituted bicyclo[2.2.2]octane-1-carboxylic acids.[11] These acids have the advantage of inflexibility so that *R* and the angle ζ are known. The fact that the carbon atoms adjacent to both the carboxyl group and the dipolar substituents are doubly substituted makes a spherical model appropriate. Using this model good agreement with experiment is obtained. It should be noted that the experimental data for these acids were measured in 50% ethanol as solvent, and that *d* values appropriate to this solvent may differ from those appropriate to water (*cf.* Discussion section).

TABLE IX

DIRECT CALCULATION OF Δ*pK* FOR BICYCLO[2.2.2]OCTANE-1-CARBOXYLIC ACIDS

	4-Br–C_8H_{12}–COOH (R = 6.50 Å.)		4-NC–C_8H_{12}–COOH (R = 7.70 Å.)	
	D_E	Δ*pK*	D_E	Δ*pK*
Observed	..	0.67	..	0.85
Calcd. vol. fixed[a] (ellipsoidal model)	6.9	.40	13	.30
Calcd. vol. fixed (spherical model)	7.6	.36	23	.17
Calcd. *d* = 1.5 Å. (ellipsoidal model)	8.4	.33	11	.35
Calcd. *d* = 1.5 Å. (spherical model)	4.6	.60	5.2	.75

[a] This calculation performed by Roberts and Moreland (ref. 11).

The preceding calculations have led to good agreement between Δ*pK* values computed on the basis of the theory and those observed experimentally. However, it is important to emphasize that to achieve this agreement required assumptions beyond those inherent in the theory, even if the fixed value of *d* is now to be accepted as part of the theory. Thus one would frequently (especially for acids with branched carbon chains) like to represent the molecule by a *lobed* cavity, but is prevented from doing so by the lack of a suitable mathematical procedure. The mathematical treatment available requires a choice between a spherical and an ellipsoidal cavity, and this choice has been shown to have a large effect on the calculated result. Furthermore, a value must be assumed for the distance *R*. It is not reasonable to expect that the simple estimation of *R* used in this paper will always correspond to the true average value of this parameter. Finally, the use of the same internal dielectric constant for all organic molecules implies equal electronic polarizability in the interior of all such molecules, which again is not necessarily correct. The resulting uncertainty in calculations of Δ*pK* is less than that inherent in the treatment used heretofore, in which *d* was allowed to vary. Nevertheless the uncertainty is still sufficiently great to make it invalid to assign differences between calcu-

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[11] J. D. Roberts and W. T. Moreland, Jr., THIS JOURNAL, **75**, 2167 (1953).

lated and observed ΔpK's to non-coulombic effects, such as "inductive" effects.[11]

Discussion

The Kirkwood model is a highly artificial representation of an organic ion. Any meaning attached to the parameter d must be a purely conventional one, somewhat like the "distance of closest approach" which occurs in the Debye–Hückel theory.

The variation of the *self-energy* of a point charge (within the cavity) with the distance d can be obtained from the complete equation for the work of charging.[12] The equation shows that the charge is repelled by the cavity and that its position of greatest stability is as close to the surface as possible. A similar conclusion applies to point dipoles. By analogy with the Debye–Hückel distance a, the distance d can thus be interpreted as a measure of the distance of closest possible approach of the solvent to a charged or dipolar site. This distance presumably represents in part the effective size of the atom or group of atoms on which a charge or dipole is centered. The effect of electric saturation of the solvent near a charged or dipolar site[13] may also be incorporated in the parameter d. In any event it is reasonable that, as a first approximation, the distance d should have about the same value for any charged site in a given solvent at a given temperature. It is also reasonable that a point dipole centered between carbon and halogen atoms should appear to be at a greater distance from the solvent than a discrete charge on an amino group or on a carboxyl oxygen atom.

To the extent that d is a measure of the physical distance from a charge or dipole to a water molecule it might be expected to *increase* with increasing temperature. It might also be expected to differ from one solvent to another. In this way one can account qualitatively for the failure of the Kirkwood–Westheimer theory, when used in such a way that d remains unaltered, to account for the effect of changing solvent or temperature upon acid dissociation constants.[14,15]

It was pointed out in the introduction to this paper that the principal objective of this investigation was to guide the extension of the Kirkwood model to globular proteins. The details of such an extension have been described in earlier papers.[16] The object was to compute titration curves of proteins, taking into account the interaction, as given by eq. 1, between all pairs of charges. Calculations were performed on simple models with structural features similar to those found in actual proteins. It was shown that calculated results agreeing with typical experimental observations could be obtained *only if d is placed equal to approximately 1 Å.* No reasonable assignment of distances between sites yields calculated titration curves resembling those obtained experimentally if d is assigned a value >1.5 Å. or <0.5 Å. This result is clearly in agreement with the conclusions reached in this paper concerning the location of charges in organic molecules in general.

Acknowledgment.—The author is pleased to acknowledge his indebtedness to Professors J. G. Kirkwood and F. H. Westheimer for helpful discussion of many aspects of this investigation.

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[12] The quantity desired is given explicitly by the term B_{kk} of ref. 16.

[13] P. Debye, "Polar Molecules," (Chemical Catalog Co.), Reinhold Publishing Corp., New York, N. Y., 1929; F. Booth, *J. Chem. Phys.*, **19**, 391 (1951).

[14] W. F. K. Wynne-Jones and G. S. Rushbrooke, *Trans. Faraday Soc.*, **40**, 99 (1944).

[15] M. Kilpatrick and J. G. Morse, THIS JOURNAL, **75**, 1846, 1854 (1953).

[16] C. Tanford and J. G. Kirkwood, *ibid.*, **79**,, 5333, 5340 (1957).

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION, CORNELL UNIVERSITY MEDICAL COLLEGE]

Studies on the Structure of Nucleic Acids. XI. The Roles of Heat and Acid in Deoxyribonucleic Acid Denaturation[1]

BY LIEBE F. CAVALIERI AND BARBARA H. ROSENBERG

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The denaturation of DNA by heat and acid has been studied by means of potentiometric titration. Titration curves have been determined at various temperatures and ionic strengths. Denaturation occurs during the steep inflection of the forward (neutrality → low pH) titration curve; the extent of denaturation depends on the proportion of this inflection which is titrated. Before denaturation begins there are some hydrogen bonds which are reversibly broken and healed. When a critical number of bonds are cleaved, denaturation sets in. This number depends on the temperature, ionic strength and the solvent system. In the presence of urea, fewer groups must be titrated potentiometrically to cause denaturation; that is, the equilibrium intact $\rightleftarrows$ broken bonds is shifted to the right for any given temperature and ionic strength. It is also concluded that the effects of heat and acid, when acting simultaneously, are additive.

The anomalous (irreversible) titration of deoxyribonucleic acid (DNA), found by Gulland and coworkers,[2] was fruitfully considered to be evidence for the existence of hydrogen bonds between the bases. However, it will be shown here that this evidence is indirect. That is, the increased pK_a''s which follow exposure to acid result directly from changes in the charge constellation of the molecule, which in turn depends on hydrogen bonding. Cox and Peacocke[3] recently have established that the back-titration curves, after exposure of DNA to pH 2.2 and pH 12.0, are coincident. This means that acid and alkali, although they directly break different hydrogen bonds since they ionize different

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[2] J. M. Gulland, D. O. Jordan and H. F. Taylor, *J. Chem. Soc.*, 1131 (1947).

[3] R. A. Cox and A. R. Peacocke, *ibid.*, 2499 (1956).