30. *Deoxypentose Nucleic Acids. Part VIII.* The Influence of Concentration and Ionic Strength on the Electrometric Titration of Sodium Deoxyribonucleate.*

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The titration curves of sodium deoxyribonucleate have been determined at different concentrations and in solutions of different ionic strengths, and a titration curve extrapolated to zero concentration and ionic strength has been obtained. These curves have been analysed and the influence of the concentration and ionic strength on the dissociation constants of the titrating groups determined. From the curve at zero concentration and ionic strength the thermodynamic dissociation constants of the titrating groups have been calculated. The electric charge carried by the nucleate ion has been calculated for solutions of different pH, concentration, and ionic strength. The influence of concentration and ionic strength on the difference between the forward and the backward titration curves has been examined.

Recent theoretical and experimental work on polyelectrolytes has shown that the apparent titration constant (pG') of a dissociating group in the polymer is strongly dependent on the concentration of the polymer and the ionic strength of the solution.[1] The results of an investigation of the effect of varying the concentration and the ionic strength of the solution on the electrometric titration of sodium deoxyribonucleate of calf thymus are reported here. In addition to the thermodynamic dissociation constants (pK_0) of the dissociating groups, it was hoped to obtain an estimate of the charge carried by the nucleate ion which could be compared with similar calculations from electrophoretic results to be reported later. A comparison of the effects of concentration and of ionic strength on the titration curves might also indicate the form of the relation between the concentration of a polyion and the ionic strength of a simple electrolyte. Also, it was hoped to obtain further information on the hydrogen bonding and intermolecular interactions in sodium deoxyribonucleate from the effect of ionic strength and concentration on the difference between the forward titration curves from the neutral region and the backward titration curves from the extremes of pH. The sample of nucleate employed [G1(ii)] has been described in Part VII (preceding paper).

Results

Effect of Ionic Strength on the Titration Curves.—Lithium chloride was used for variation of the ionic strength of the nucleate solutions because its high solubility made it possible to raise the ionic strength (I) of the solutions without greatly altering their volumes. A series of solutions of sodium deoxyribonucleate of the same concentration (0·00142 equiv. of 4P per l.) were brought to pH 12·00 by the addition of 0·5N-sodium hydroxide, and then different amounts of 0·5N-hydrochloric acid were added to give the solutions different pH values in the range pH 3—12. These solutions were then titrated with 12·228M-lithium chloride; the results, plotted as pH versus $\sqrt{I}$, are shown in Fig. 1. Titration curves at constant ionic strength may be obtained from these curves, which describe the effect of ionic strength on the backward titration curve of sodium deoxyribonucleate. A few similar results on the forward titration curve are shown in Fig. 2. For these experiments the initial addition of sodium hydroxide to bring the solutions to pH 12 was omitted. No evidence was obtained that lithium chloride influences the hydrogen bonds. By titration with lithium chloride rather than with acid or alkali, titration curves at constant ionic strength were obtained by rapid continuous titration, instead of having to make up the solution separately for each point on the titration curve. Also, it disposed of difficulties associated with the precipitation of the nucleate in salt solution on the addition of acid.

* Part VII, preceding paper.

[1] Katchalsky *et al.*, *J. Polymer Sci.*, 1947, **2**, 432; 1950, **5**, 283; 1954, **12**, 159; Arnold and Overbeek *Rec. Trav. chim.*, 1950, **69**, 192.

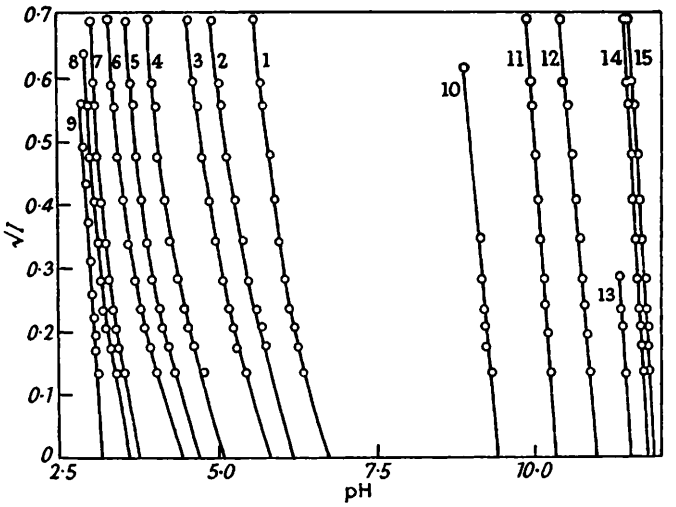

FIG. 1. *Effect of ionic strength on the* pH *of nucleate solutions on backward titration.*

Normality of HCl *present* :

1, 0;
2, 0·00025;
3, 0·0005;
4, 0·001;
5, 0·0015;
6, 0·002;
7, 0·0025;
8, 0·003;
9, 0·004.

Normality of NaOH *present* :

1, 0;
10, 0·00075;
11, 0·002;
12, 0·004;
13, 0·005;
14, 0·0095;
15, 0·0125.

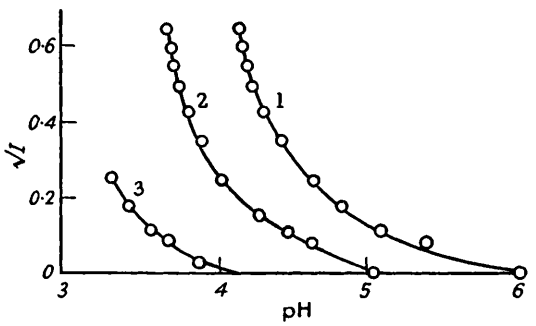

FIG. 2. *Effect of ionic strength on the* pH *on forward titration on the acid side.*

Normality of HCl *present* :

1, 0;
2, 0·0005;
3, 0·002.

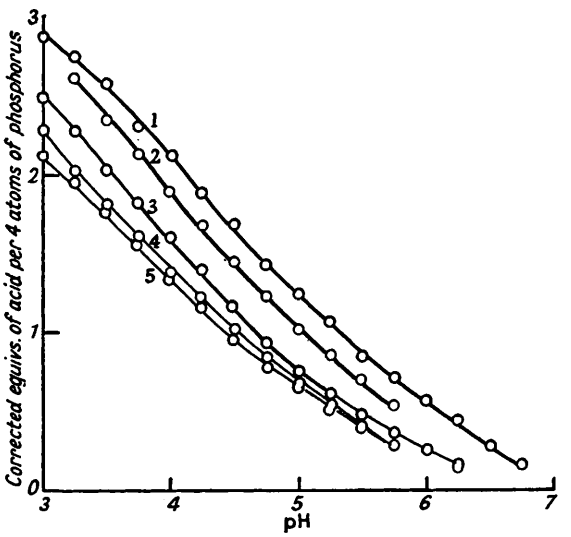

FIG. 3. *Backward titration curves in the acid region at different nucleate concentrations* (*C*).

1, $C = 1{\cdot}13$;
2, $C = 1{\cdot}88$;
3, $C = 2{\cdot}81$;
4, $C = 3{\cdot}75$;
5, $C = 11{\cdot}25$ (*C in* 10^{-4} *equiv. of* 4P *atoms per l.*).

Effect of Concentration on the Titration Curves.—Sodium deoxyribonucleate solutions of concentration 0·26—0·016% were titrated to pH 2 with 0·05N- or 0·5N-hydrochloric acid, and back to neutrality with 0·05N- or 0·5N-sodium hydroxide, Fig. 3 shows some of the backward titration curves from pH 2 obtained. The corresponding forward curves are shown in Fig. 1 of Part VII. The lowest concentration at which accurate results could be obtained was 0·016%.

Effect of Concentration and Ionic Strength on the Hysteresis.—The same curves were used to obtain the effect of concentration on the difference between the forward and the backward titration curves, but to find the influence of ionic strength on this hysteresis effect, sodium deoxyribonucleate in concentrated aqueous potassium chloride was titrated to pH 12 and back to neutrality. This could not be done on the acid side.

Analysis of the Titration Curves.—The backward titration curves at different ionic strengths and concentrations were analysed by the method used in Part VII to obtain the pG' values of the dissociating groups. The analytical quantities of guanine, adenine, cytosine, and thymine present in the nucleate were taken as 0·86, 1·13, 0·85, and 1·11 mole per 4 g.-atoms of phosphorus, respectively (cf. Part VII). Titration curves were obtained for zero ionic strength at a concentration of nucleate of 0·00142 equiv. of 4P per l. by extrapolation of the plots of pH against $\sqrt{I}$, and for zero concentration and zero ionic strength by plotting concentration against the amount of acid or alkali used in titration at different pH values and taking the slopes at the origin. These extrapolated titration curves are shown in Fig. 4. They were also analysed

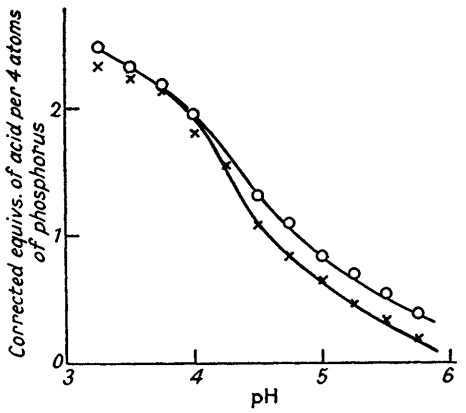

FIG. 4. *Titration curve of sodium deoxyribonucleate at zero concentration and ionic strength.*

× *Forward titration.* ○ *Backward titration.*

for the pG' values of the dissociating groups. The pG' values of the titrating groups at various concentrations and ionic strengths, and the calculated amount of secondary phosphoryl dissociation, are collected in Table 1.

The presence of a secondary phosphoryl dissociation was inferred by Gulland, Jordan, and Taylor [2] and by Lee and Peacocke [3] because of the necessity of introducing a small quantity of a group dissociating between pH 6 and 7 to explain their titration curves. This is also necessary to explain the present results. However, the forward titration curves (see Part VII) show no indication of the presence of such a group, and there is no evidence for a secondary phosphoryl dissociation from other sources. It cannot be considered definitely established that this group is a secondary phosphoryl dissociation. Lawley [4] has suggested that it may be due to an amino-dissociation modified by hydrogen bonding with primary phosphate groups. For 12 of the 13 backward titration curves analysed, the amount of this dissociation found was 0·19—0·21 equiv. per 4P atoms. This is less than that reported by Lee and Peacocke,[3] namely, 0·33 ± 0·07 equiv. for undried material calculated from the backward titration curve from pH 2, but is in agreement with the conclusions of Gulland, Jordan, and Taylor [2] who found 0·25 equiv., although it must be remembered that they employed incorrect analytical figures.

The Dissociation Constants.—The titration constants (pG') are evaluated by the equation $\mathrm{pH} = \mathrm{p}G' - \log [\beta/(1 - \beta)]$ where β is the degree of dissociation of the base concerned.

[2] Gulland, Jordan, and Taylor, *J.*, 1947, 1131.
[3] Lee and Peacocke, *J.*, 1951, 3361.
[4] Lawley, *Biochim. Biophys. Acta*, 1955, in the press.

Katchalsky, Shavit, and Eisenberg [5] have shown that the dissociation of a polymeric electrolyte may be represented by :

$$\mathrm{pH} = \mathrm{p}K_0 - \log \frac{\beta}{1-\beta} - \frac{0{\cdot}4343e\psi}{kT}$$

where ψ is the electrostatic potential which may be evaluated from electrophoretic measurements. The term involving ψ is not negligible for simple polyelectrolytes. A study of the electrophoretic mobility of sodium deoxyribonucleate has been made at various pH values and ionic strengths, and the magnitude of the term $\frac{0{\cdot}4343e\psi}{kT}$ has been calculated. It accounts correctly for the departure of the value of pG' from pK_0 at finite concentrations and ionic strengths (see Part IX, following paper).

TABLE 1. *Dissociation constants of sodium deoxyribonucleate at different concentrations and ionic strengths.*

Concn. of nucleate in equiv. 4P per l.	Ionic strength	pG'				Equiv. *sec.*-P(?) per 4P
		Guanine	Adenine	Cytosine	*sec.*-P(?)	
0	0	3·45	4·25	5·25	—	0·20
0·000113	—	3·35	4·20	5·25	6·45	0·21
0·000188	—	3·35	4·15	5·20	6·1	0·26
0·000281	—	2·95	3·90	5·10	6·2	0·20
0·000375	—	2·70	3·75	4·95	—	0·20
0·000750	—	2·50	3·70	4·90	6·1	0·19
0·001125	—	2·40	3·65	4·85	—	0·21
0·00142	0	2·60	4·05	5·25	5·90	0·21
,,	0·133	1·90	2·90	4·15	5·25	0·20
,,	0·255	1·70	2·60	4·05	5·10	0·20
,,	0·368	1·60	2·50	3·95	5·00	0·20
,,	0·492	1·50	2·50	3·95	4·75	0·20
,,	0·622	1·40	2·35	3·65	4·90	0·20
,,	0·748	<1·4	2·30	3·60	4·80	0·20
,,	0·871	<1·4	2·20	3·55	4·75	0·20

DISCUSSION

The values of pG' at zero concentration and ionic strength are the thermodynamic dissociation constants (pK) of the bases, and they are shown in the first line of Table 1. Increase in concentration and ionic strength both result in a smooth decrease in the pG' values, which becomes less marked at both high concentrations and high ionic strengths. The pG'–concentration curves however show an inflection at low concentrations not observed in the pG'–ionic strength curves. The effect of concentration is greatest on the pG' of guanine and less on the pG' of the groups titrating at higher pH, but the effect of ionic strength is least on the pG' of guanine.

Comparison of the Effects of Concentration and Ionic Strength.—Although concentration and ionic strength affect the pG' values in a similar manner, the ionic strength of the solution has a much greater effect on the pG' of the bases than has the concentration of nucleate, but the nucleate concentrations expressed in equivs. of 4P atoms per l., or as molar concentrations, are very small. For equal molar concentrations of nucleate and simple ion, the effect of the nucleate would be very much greater. Rough correspondence is shown between the ionic strength and the nucleate concentrations expressed as a percentage, but concentration has a relatively greater effect at low pH while ionic strength has the greater influence at higher pH. An attempt was made to estimate the " effective ionic strength " of sodium deoxyribonucleate at neutral pH, by titrating phosphoric acid in its presence. The titration curves over the neutral region, where the secondary phosphate groups are being titrated, were identical in the presence of and absence of 0·25% of sodium deoxyribonucleate, but small concentrations of added salt produced measurable differences. Clearly the nucleate exerts no measurable ionic-strength effect under these conditions and is less likely to do so at lower pH when its net charge is smaller.

[5] Katchalsky, Shavit, and Eisenberg, *J. Polymer Sci.*, 1954, **13**, 69.

Effect of Concentration and Ionic Strength on the Hysteresis.—The ionic strength of the solution has no effect on the alkaline hysteresis, measured by the area between the forward and the backward titration curves between pH 10 and 12 (Table 2). The acid hysteresis,

TABLE 2. *Effect of* KCl *on the alkaline hysteresis between* pH 10 *and* 12, *concn.* 0·25%.

Concn. of KCl (M)	0	0	0·1	0·25	1·00
Area of hysteresis (arbitrary scale)	47·5	46·2	47·0	48·5	46·5

as measured by the area berween the forward and the backward titration curves between pH 4 and 7, is directly proportional to the nucleate concentration (Fig. 5). Within experimental error, hysteresis always begins at pH 4·0, irrespective of the nucleate concentration. Clearly the forward and the backward titration curves are both influenced to the same extent by the ionic strength of the solution, and by the concentration of the nucleate. This suggests that hysteresis is not due to electrostatic forces, or to intermolecular interaction, but to some molecular property such as the intramolecular hydrogen bonding, to which it has already been ascribed.

The Charge carried by the Nucleate Ion.—The titration curves may be used to calculate the net negative charge borne by the nucleate ion. The calculations are strictly valid

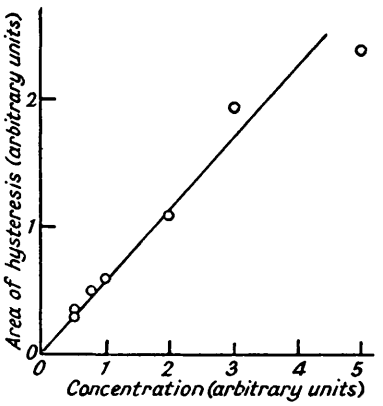

FIG. 5. *The effect of concentration on the hysteresis.*

only for a titration curve extrapolated to zero concentration and ionic strength [6] such as has been determined. The value of the charge so obtained will refer to a nucleate ion for which there has been no absorption of gegenions.

If $7{\cdot}9 \times 10^6$ is taken for the molecular weight (Part VII), and 1231 for the molecular weight per 4P atoms, the maximum number of positive charges which are realisable when all the bases are titrated is 18,200. The number of positive charges on the nucleate ion has been calculated from the titration curve at zero concentration and ionic strength at various pH's and this is shown in Table 3. The net charge will be determined by the

TABLE 3. *Charge carried by the nucleate ion in the absence of gegenions.*

pH	3·0	3·5	4·0	4·5	5·0	5·5	6·0
No. of positive charges	15,100	13,600	11,200	6750	4240	2120	578
Net negative charge	10,580	12,080	14,480	18,930	21,449	23,560	25,102

number of positive charges, and by the number of negative charges carried by the primary phosphate groups. At pH values greater than 3 the primary phosphate groups may be considered to be fully dissociated and so the maximum negative charge is 25,680. The net negative charge on the nucleate ion is also shown in Table 3.

In practice at finite concentration and ionic strength, a number of Na^+ ions will be

[6] Abramson, Moyer, and Gorin, " Electrophoresis of Proteins," Reinhold Publ. Corpn., New York, 1942, pp. 156 *et seq.*

bound by ion-pair formation with the primary phosphate groups, and so the total negative charge is likely to be considerably less than 25,680; furthermore, Cl^- ions may form ion-pairs with the charged amino-groups, and so the total positive charge will also be less than that shown in Table 3. The change in the titration curves with concentration and ionic strength is to be ascribed partly to the influence of the bound gegenions on the electrostatic potential of the nucleate ion, as well as to the effect of the ionic strength on the activity coefficients. The net negative charge on the nucleate ion including its gegenions will therefore depend on the relative binding of the Na^+ and Cl^- gegenions, as well as on the degree of dissociation of the amino-groups. Values of the net negative charge calculated from the titration curves at various concentrations and ionic strengths, shown in Table 4, may allow for the effect on the activity coefficients of the simple ions, but take no account of the effect of absorption of the gegenions.

The figures in Table 4 may be compared with values of the net charge calculated by methods which allow for the contribution of the gegenions to it. For this sample of deoxyribonucleic acid, the value of the net negative charge calculated from electrophoretic mobility measurements (see Part IX, following paper) lies between 1500 and 2800, which is of the same order of magnitude as the values given by Creeth and Jordan [7] and Shack,

TABLE 4. *Net negative charge on the nucleate ion at various concentrations and ionic strengths, the contributions of the gegenions being disregarded.*

	Concn. 0·00142 equiv. of 4P per litre						Absence of added salt		
pH	$\sqrt{I}$: 0	0·1	0·2	0·3	0·5	0·7	c : 0·001125	0·000375	0·000281
6	25,420	—	—	—	—	—	25,680	25,680	25,360
5·5	23,880	24,780	25,420	—	—	—	24,530	24,390	23,940
5	21,830	23,240	24,010	24,650	25,490	—	22,920	22,600	22,080
4·5	19,310	20,730	22,080	23,120	24,070	24,720	20,930	20,480	19,010
4	15,440	17,980	19,110	20,540	21,960	22,920	18,500	17,910	16,680
3·5	12,780	14,580	15,680	17,050	18,580	19,560	15,800	15,280	13,930
3	—	—	—	—	14,080	15,480	13,480	12,180	10,880

Jenkins, and Thompsett.[8] It is thus evident that a large proportion of the charge of the nucleate ion itself is neutralised by the binding of gegenions of opposite charge.

The effects of concentration and of ionic strength on the net charge, the gegenions being disregarded, are similar, as shown by the figures in Table 4. This is to be expected from the similarity in the effects of concentration and ionic strength on the pG' of the bases.

Experimental.—The preparation and properties of the sodium deoxyribonucleate, the preparation of the solutions for titration, and the titration procedure were as in Part VII, except that in the experiments on the effect of ionic strength, solutions of the nucleate containing varying amounts of acid or alkali were titrated with concentrated lithium chloride solution.

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[7] Creeth and Jordan, *J.*, 1949, 1409.
[8] Shack, Jenkins, and Thompsett, *J. Biol. Chem.*, 1952, **198**, 85.