62. *Deoxypentose Nucleic Acids. Part IX.* The Electrophoretic Mobility of Sodium Deoxyribonucleate at Various* pH *Values and Ionic Strengths.*

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The electrophoretic mobility of sodium deoxyribonucleate has been measured in solutions of ionic strength 0·02—0·2 and pH 3·5—7·0. The net charge on the nucleate ion and its associated gegenions has been calculated from these results and compared with the charge on the nucleate ion itself calculated from electrometric titration data. An estimate has been made of the numbers of sodium and chloride gegenions bound to the nucleate ion at the different pH values and ionic strengths. The titration constants of the bases calculated from the titration curves have been shown to be equal to the dissociation constants of the bases in the nucleic acid molecule.

A NUMBER of investigations of the electrophoretic mobility of sodium deoxyribonucleate have been reported.[1] It was thought desirable to repeat this work, some of which was done on more or less degraded material, and to extend it to lower values of pH. The electrophoretic mobility of undegraded sodium deoxyribonucleate of high molecular weight was measured at various pH's and ionic strengths, particularly over the pH range 3—7 where the amino-groups of the purine and pyrimidine bases are being titrated, with consequent rapid alteration in the charge on the nucleate ion. The sample employed, designated G1(II), was sodium deoxyribonucleate of calf thymus extracted by the method of Gulland, Jordan, and Threlfall,[2] being the sample for which the electrometric titration data had been obtained.[3] It was of high molecular weight ($7·9 \times 10^6$ by sedimentation and diffusion [4]) and has been fully described.[3a] This sample is probably better than that used in the earlier study by Creeth, Jordan, and Gulland.[1b]

RESULTS

This sample of sodium deoxyribonucleate migrated as a single boundary under all the conditions of pH and ionic strength studied. Both the boundaries remained sharp during electrophoresis, particularly the ascending boundary. The mobility calculated from the ascending boundary was never more than 2% greater than that calculated from the descending boundary, and so the average of the two values was taken to be the true electrophoretic mobility. No δ or ε boundary was observed, perhaps owing to the relatively low concentration of the nucleate ion and the relatively high concentrations of added salt.

Variation of Mobility with pH *and Ionic Strength.*—The mobilities (μ), determined at different pH's and ionic strengths (I), are plotted in the Figure against I for the various pH values. The ionic strength of the solutions was varied by using sodium chloride as the added electrolyte, and the nucleate concentration was 0·20% in all the experiments. This was not varied, since Creeth, Jordan, and Gulland [1b] showed that the mobility was little affected by the concentration over the range which can be studied in electrophoresis. In view of the effect of concentration on the electrometric titration results, which is only significant [3b] at concentrations below 0·03%, it may be that at these low concentrations the mobility is appreciably influenced by the concentration; clearly the mobility results may not be extrapolated to zero concentration. The extrapolation of the mobilities to zero ionic strength is uncertain at the higher pH values.

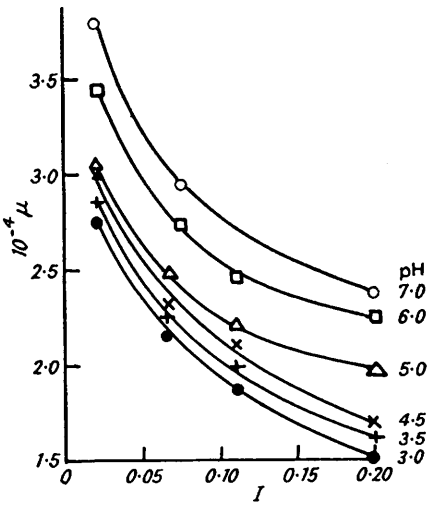
* Part VIII, *J.*, 1956, 158.

[1] (*a*) Stenhagen and Teorell, *Trans. Faraday Soc.*, 1939, **35**, 743; Seibert, *J. Biol Chem.*, 1940, **133**, 593; Hall, *J. Amer. Chem. Soc.*, 1941, **63**, 794; Zittle and Seibert, *J. Immunol.*, 1942, **43**, 47; Cohen, *J. Biol Chem..*, 1942, **146**, 471; (*b*) Creeth, Jordan, and Gulland, *J.*, 1949, 1406 (Part IV), 1409 (Part V).
[2] Gulland, Jordan, and Threlfall, *J.*, 1947, 1129.
[3] Jordan, Mathieson, and Matty, *J.*, 1956, (*a*) 154 (Part VII), (*b*) 158 (Part VIII).
[4] Howard, Thesis, Nottingham, 1953.

Net Charge carried by the Nucleate Ion.—The net charge carried by the nucleate ion and its associated gegenions (Q) can be calculated by Gorin's equation : [5b]

$$\mu = \frac{2Q}{(l + 2a)F'(\kappa a)\pi\eta}\left[\frac{K_0(\kappa a + \kappa r_i)}{(\kappa a + \kappa r_i) \cdot K_1(\kappa a + \kappa r_i)} + \ln\left(\frac{a + r_i}{a}\right)\right]$$

where l and a are respectively the length and radius of the cylindrical colloid particle, r_i is the average radius of the ions in the ionic atmosphere, κ is the reciprocal Debye–Hückel radius, $F'(\kappa a)$ is a function to account for the random orientation of the particles, η is the coefficient of viscosity of the solvent, and K_0 and K_1 are Bessel functions. Values of $F'(\kappa a)$, K_0, and K_1



The electrophoretic mobility at various pH values and ionic strengths.

have been tabulated by Abramson, Moyer, and Gorin.[5b] The length of the nucleate ion is still somewhat uncertain, and the values shown in Table 1 have been employed, based on the results of Rowen [6] and Steiner [7] and the model of a flexible rod. The radius was calculated by using the value 0·50 determined for the partial specific volume, and the molecular weight $7·9 \times 10^6$. The value of 2·24 Å was used for r_i this being the mean of the value for the sodium

TABLE 1. *Dimensions of the nucleate ion.*

I	0·02	0·05	0·10	0·20
Length (l) (Å)	5300	4900	4700	4500
Radius (a) (Å)	19·6	20·6	21·1	21·6

TABLE 2. *The net negative charge carried by the nucleate ion and its associated gegenions.*

	I					
pH	0·02	0·05	0·10	0·15	0·20	0·25 *
3·0	1970	2020	2020	1930	1830	1730
3·5	2040	2100	2030	2000	1990	1940
4·5	2140	2220	2260	2170	2080	2030
5·0	2190	2330	2380	2410	2420	2430
6·0	2460	2620	2700	2730	2760	2780
7·0	2710	2820	2910	2910	2900	2900

* Extrapolated values.

and the chloride ions.[8] κ at 0·5° is $0·323 \times 10^8\sqrt{I}$, and η at this temperature is $1·76 \times 10^{-2}$ poise. The values of the net charge Q at the various pH and ionic strengths are shown in Table 2.

[5] (*a*) Abramson, Gorin, and Moyer, *Chem Rev.*, 1939, **24**, 345; (*b*) Abramson, Moyer, and Gorin, "Electrophoresis of Proteins," Reinhold Publ. Corpn., New York, 1942.
[6] Rowen, *Biochim. Biophys. Acta*, 1953, **10**, 391.
[7] Steiner, *Trans. Faraday Soc.*, 1952, **48**, 1185.
[8] Gorin, *J. Chem. Phys.*, 1939, **7**, 405.

Discussion

Electrophoretic Mobility and Net Negative Charge.—The Figure shows that μ is decreased by increase in the ionic strength and by lowering the pH of the solution. The decrease in μ at lower pH will be due primarily to the ionisation of the amino-groups, with consequent reduction in the net negative charge on the nucleate ion. The decrease of μ with increase of ionic strength will be due to the increase of interionic attractive forces, and perhaps partly to an increase in the proportion of bound sodium gegenions. At higher ionic strengths the pG' values of the purine and the pyrimidine bases are decreased [3b] and this would lead to an increase of charge with I at the lower pH values. Clearly this effect is masked by the other two. The net negative charge increases sharply with ionic strength up to I ~0·10, thereafter either decreasing (pH 3—4·5) or increasing only very slightly (pH 5—7), and the decrease in the pG' values at higher ionic strengths may be responsible for this. Decrease of pH always leads to a reduction in the net negative charge. The values of the net negative charge depend markedly on the length taken for the nucleate ion.

Comparison of Electrophoretic and Titration Results: Proportion of Bound Gegenions.—The values of the net negative charge (Q_0) on the nucleate ion itself calculated from the results of titration [3b] may be compared with those of the net negative charge (Q) carried by the nucleate ion and its associated gegenions at various pH's and ionic strengths. The difference between these two quantities ($Q_0 - Q$) should be equal to the net positive charge of the bound gegenions, *i.e.*, ($Q_{Na^+} - Q_{Cl^-}$), where Q_{Na^+} and Q_{Cl^-} are the numbers of bound sodium and chloride gegenions, respectively, per nucleate ion.

The value of ($Q_0 - Q$) (Table 3) increases with increase of pH and ionic strength. At pH 6·0 it is a maximum and is virtually constant, decreasing very slightly with increase of I.

TABLE 3. *The binding of gegenions.*

	$(Q_0 - Q) = Q_{Na^+} - Q_{Cl^-}$						Q_{Cl^-}					
pH \ I	0·02	0·05	0·10	0·15	0·20	0·25	0·02	0·05	0·10	0·15	0·20	0·25
3·5	13,060	14,090	15,080	15,700	16,110	16,660	9940	8910	7920	7300	6890	6340
4·5	19,160	20,080	20,940	21,430	21·910	22,170	3840	2920	2060	1570	1090	830
5·0	21,400	21,930	22,420	22,690	22,990	23,060	1600	1070	580	310	10	0
6·0	23,180	22,990	22,940	22,880	22,860	22,810	0	0	0	0	0	0

Since there are no positively charged sites on the nucleate ion at this pH at the higher ionic strengths, there can be no chloride gegenions directly attached to the nucleate ion. The number of bound sodium gegenions (Q_{Na^+}) when there are no positive sites on the nucleate ion is therefore approximately 23,000, and this is ~90% of the total number of primary phosphoryl groups (25,680). The same proportion has been found from spectroscopic studies.[9] If a significant proportion of chloride ions were bound by the sodium gegenions, Q_{Na^+} would have to be even greater than 23,000 and this seems unlikely. At pH values greater than 3·0 the primary phosphate groups are all ionised, and so the effect of changing the pH from 3·5 to 6·0 on the number of bound sodium ions is not likely to be great. To a first approximation, then, Q_{Na^+} can be taken as 23,000 for all the pH values. The number of chloride ions bound to the charged amino-groups per nucleate ion (Q_{Cl^-}) can therefore be calculated, and these results are also shown in Table 3. The values of Q_{Cl^-} are quite reasonable, increasing rapidly as the pH is lowered and the number of charged amino-groups increased. The proportion of the amino-groups which are ionised at a given pH and ionic strength may be calculated from the pG' values of the bases given in Table 1 of the preceding paper, and comparison with the values of Q_{Cl^-} in Table 3 then shows that the fraction of charged amino-groups which have a bound chloride ion increases rapidly as the pH is lowered, becoming just less than unity at pH 3·5. This is similar to the behaviour of polyacrylic acid with sodium gegenions when the degree of ionisation of the acid is increased.[10]

[9] Lawley, personal communication.
[10] Huizenga, Greiger, and Wall, *J. Amer. Chem. Soc.*, 1950, **72**, 2636.

The net charge on the nucleate ion is always negative. The possibility exists therefore that this might preclude the binding of chloride ions owing to electrostatic repulsion. However, the binding of chloride ions to the deoxyribonucleate ion has been observed by Shack, Jenkins, and Thompsett [11] who concluded, from membrane potential measurements and chemical analysis after dialysis equilibrium, that 0·32 ion of chloride was bound per four atoms of phosphorus. Moreover, if no chloride ion is bound it must be assumed that a decrease of bound sodium ion occurs on increase of sodium-ion concentration, to explain their results. No evidence of this was found.

It is also known that chloride ions are bound by certain proteins. Thus Alberty and Marvin's results [12] show that the chloride ions are bound to bovine serum albumin between pH's of 7·0 and 3·2, although the isoelectric point of this protein is 4·9.

Comparison of Electrophoretic and Titration Results: Dissociation Constants.—Katchalshy, Shavit, and Eisenberg [13] have shown that the titration of a weak polymeric electrolyte can be represented by the expression

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

where β is the degree of dissociation and ψ is the electrostatic potential. Only if the term involving ψ is negligible can the titration constants of the bases (pG'), given by pH = pG' $-$ log[β/(1 $-$ β)], be identified with the dissociation constants (pK_0) of the bases in the nucleate. This term is not negligible for simple polyelectrolytes.

The value of ψ can be calculated from the electrophoretic data. ψ is related to the electrophoretic mobility by the equation $\psi = 300\ \eta\mu/CD$, where D is the dielectric constant of the medium, and C is a factor which depends on the shape and orientation of the migrating ion. If the nucleate ion can be treated as a long cylinder orientated randomly with respect to the field, $C = 1/\pi\mathrm{F}'(\kappa a)$ (Abramson, Moyer, and Gorin [5b]). Table 4 shows the values of ψ and of $0{\cdot}4343e\psi/kT$ for various pH values at $I = 0{\cdot}10$.

TABLE 4. *The electrostatic potential* ($I = 0{\cdot}10$).

pH	3·0	3·5	4·5	5·0	6·0	7·0
$10^4\psi$	2·23	2·35	2·47	2·56	2·90	3·20
$0{\cdot}4343e\psi/kT$	1·14	1·20	1·26	1·30	1·48	1·63

The term $0{\cdot}4343e\psi/kT$ varies only slightly with pH (only 0·1 pH unit for a change of pH of unity) and so causes no appreciable spread of the titration curves. This observation, which explains the success of Gulland, Jordan, and Taylor [14] in identifying the titration constants of nucleic acid with the bases concerned, is different from the behaviour of synthetic polyelectrolytes. It may be that the presence of the large gegenion atmosphere and the overlapping nature of the dissociations concerned are responsible for this. The magnitude of $0{\cdot}4343e\psi/kT$ accounts correctly for the departure of the value of pG' from pK_0 at finite concentrations and ionic strengths.

EXPERIMENTAL

Preparation and Analysis of the Solutions.—Undried sodium deoxyribonucleate was dissolved in water without stirring to give a stock solution of twice the desired concentration, which was kept at 0°. Samples of this solution were diluted with equal volumes of buffer solutions of twice the desired ionic strength. These solutions were then dialysed against the appropriate buffer solution for 17 hr. The buffer solutions contained sodium chloride to give the desired ionic strength, and virtually all the ionic strength of the buffer solutions was due to this. The buffer solutions [15] were: glycine–hydrochloric acid for pH 3·5, acetate for pH 4·5 and 5·0, and phosphate for pH 6·0 and 7·0.

[11] Shack, Jenkins, and Thompsett, *J. Biol. Chem.*, 1952, **198**, 85.
[12] Alberty and Marvin, *J. Amer. Chem. Soc.*, 1951, **73**, 3220.
[13] Katchalsky, Shavit, and Eisenberg, *J. Polymer Sci.*, 1954, **13**, 69.
[14] Gulland, Jordan, and Taylor, *J.*, 1947, 1131.
[15] Cf. Miller, *Arch. Biochem.*, 1950, **29**, 420.

The nucleate concentration of the stock solution was determined by the method of Jones, Lee, and Peacocke.[16]

Electrophoretic Mobility.—The mobilities were determined at 0·5° in a modified form of the Tiselius electrophoresis apparatus [17] constructed by Adam Hilger, Ltd. The methods used were those described by Creeth, Jordan, and Gulland.[1b] The conductivities were determined by the use of a Mullard conductance bridge, and the pH of the solutions was measured electrometrically to ±0·01 pH unit with a Tinsley potentiometer, a standard Weston cell, a hydrogen electrode, and a standard silver–silver chloride electrode.

We express our appreciation to the British Empire Cancer Campaign (Nottinghamshire Branch) for financial help and the award of a bursary (to J. V. M.), to Imperial Chemical Industries Limited for the loan of apparatus, and to Sheila Matty, B.Sc. for help with some of the experiments.

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[16] Jones, Lee, and Peacocke, *J.*, 1951, 623.
[17] Tiselius, *Trans. Faraday Soc.*, 1937, **33**, 524.