300. *Deoxypentose Nucleic Acids. Part V. An Attempted Interpretation of the Electrophoretic Mobilities of the Deoxypentose Nucleic Acid of Calf Thymus.*

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The charge on the deoxypentose nucleate ion in solutions of various ionic strengths has been determined from membrane potential measurements. The value of the charge is found to be considerably lower than that calculated from the analytical and molecular-weight data, and the reason for this is discussed. From the values of the particle charge, the electrophoretic mobility has been calculated from the equation of Gorin (Abramson, Gorin, and Moyer, *Chem. Reviews*, 1939, **24**, 345) for long cylinders. Agreement between the calculated and observed values is found to be excellent at low values of the ionic strength, but at higher values considerable discrepancies occur; possible explanations for this behaviour are put forward.

During an investigation of the electrophoretic behaviour of the sample of the sodium salt of the deoxypentose nucleic acid of calf thymus (Creeth, Jordan, and Gulland, Part IV, preceding paper) which had previously been studied by electrometric titration (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131) and by viscosimetric methods (Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141), a large variation of the mobility of the deoxypentose nucleate ion with change of ionic strength was observed. The molecules of this nucleic acid have been shown to be cylindrical in shape with a large axial ratio (see, *e.g.*, Signer, Caspersson, and Hammarsten, *Nature*, 1938, **141**, 122; Astbury, *Symp. Soc. Exp. Biol.*, 1946, **1**, 66; Cecil and Ogston, *J.*, 1948, 1382), and furthermore it has been shown that the particular sample of this nucleic acid referred to above is homogeneous as to molecular size and weight (Cecil and Ogston, *loc. cit.*). It was therefore considered that the electrophoretic data obtained by Creeth, Jordan, and Gulland (*loc. cit.*) presented an opportunity of testing the present electrokinetic theory of long cylindrical particles, and possibly of gaining further light on the unusual physical properties of solutions of the sodium salt of the deoxypentose nucleic acid of calf thymus, *e.g.*, the very high viscosity.

Few attempts have previously been made to interpret the observed mobility characteristics of biocolloids. The work of Tiselius and Svensson (*Trans. Faraday Soc.*, 1940, **36**, 16) on egg albumin, which demonstrated a close correspondence between the observed and cal-

culated mobilities over a range of ionic strength, has been criticised by Gorin (*J. Physical Chem.*, 1941, **45**, 371), who considered the refinements to the Henry–Debye–Hückel theory which should be applied. Adair and Adair (*Trans. Faraday Soc.*, 1940, **36**, 23) also obtained satisfactory agreement between the observed and calculated mobilities of hæmoglobin by the use of the uncorrected Henry–Debye–Hückel equation. In both these cases the protein molecules were assumed to be spherical. Gorin and Moyer (*J. Gen. Physiol.*, 1942, **25**, 785) have applied a theoretical equation for the mobilities of cylindrical particles to the data for serum albumin-*B*. Good agreement was obtained between the particle charge values calculated from mobilities and those calculated from electrometric titration data over a small range of pH close to the isoelectric point. The asymmetry of the molecules of this protein is, however, small.

The most satisfactory method of testing the validity of electrokinetic equations is to obtain values of the particle charge by an independent means and then to calculate theoretical mobilities. Such particle charge values may, in certain cases, be determined from the acid–base binding curve of the substance; the problem is complicated, however, by the binding of ions other than hydrogen or hydoxyl, and Abramson, Moyer, and Gorin ("The Electrophoresis of Proteins," Reinhold Publishing Corporation, 1942) have concluded that, for proteins, accurate values of the particle charge can only be obtained from titration measurements which have been extrapolated to zero ionic strength and zero protein concentration. This method clearly cannot give values of the particle charge over a range of ionic strengths, and we have therefore employed the method described by Tiselius and Svensson (*loc. cit.*) and Adair and Adair (*loc. cit.*) in which the particle-charge values are obtained from membrane potential measurements. The membrane potential is the potential existing between a colloidal solution and a buffer solution from which it is separated by a membrane, permeable to the electrolyte ions but impermeable to those of the colloid, the two solutions being in Donnan equilibrium. This method, initiated by Loeb ("Proteins and the Theory of Colloidal Behaviour," New York, 1922) and developed by Adair and Adair (*Biochem. J.*, 1934, **28**, 199; *loc. cit.*), gives values for the equivalent concentration of the colloidal ion, and thus, if the molecular weight is known, the particle charge can be determined. By this method it is possible to obtain values of the particle charge under conditions of different ionic strength.

Results.—The results of the determination of the particle charge of the deoxypentose nucleate ion are shown in Table I. The values of the equivalent concentration (c) of the deoxypentose nucleate ion were calculated from the equation

$$c = [\text{sum of equivalents of cations}]_2 - [\text{sum of equivalents of anions}]_2$$

where the terms within the brackets and designated by the subscript 2 refer to the concentrations of the solution within the membrane containing the colloidal ion. These concentrations are related to those outside the membrane (designated by the subscript 1) by the equations

$$[\text{cations}]_2 = f[\text{cations}]_1$$

and

$$[\text{anions}]_2 = [\text{anions}]_1/f$$

where f is the ideal distribution ratio for univalent ions and is related to the membrane potential (E) by the equation

$$E = -\frac{RT}{F}\ln f$$

The above ideal equations may only be applied where (i) the liquid junction potentials, KCl/solution 1 and KCl/solution 2 are equal and (ii) the activity coefficients of the ions on both sides of the membrane are equal. It is general to assume (i) to be correct, and Adair and Adair (1934, *loc. cit.*) have shown that if the membrane potential is small (ii) may also be assumed without serious error.

The values of the particle charge were calculated from the equivalent concentration data, the value $8{\cdot}2 \times 10^5$ for the molecular weight of the deoxypentose nucleate ion (Cecil and Ogston, *loc. cit.*) being used.

The values given in Table I, which have been obtained by the approximate elimination of liquid junction potentials by means of concentrated potassium chloride, must be regarded as conventional (Adair and Adair, *loc. cit.*). Moreover in view of the small magnitude of most of the membrane potentials and the errors inherent in the method, the results, particularly at the higher ionic strengths, can only be regarded as approximate. The great interest, however, attached to even approximate data renders the method of value.

TABLE I.

The charge on the deoxypentose nucleate ion at pH 7.

Concn. of deoxypentose nucleate ion (%).	Composition of buffer.	Ionic strength (I).	Membrane potential (E) (mv.).	$c \times 10^3$.	Charge (xe^{-1}).
0·8	NaCl	0·20	0·63	10·70	1090
0·8	NaCl	0·20	0·54	8·10	(830)
0·6	NaCl	0·20	0·54	8·10	1100
0·6	NaCl	0·08	0·95	6·47	882
0·6	Sodium phosphates	0·05	1·19	5·11	698
0·6	NaCl	0·02	2·67	4·50	615
0·6	Sodium phosphates	0·01	4·80	3·70	508
0·6	NaCl	0·005	8·59	3·73	509

Values of the electrophoretic mobility can be calculated by the equation developed by Gorin (see Abramson, Gorin, and Moyer, *Chem. Reviews*, 1939, **24**, 345; Abramson, Moyer, and Gorin, *loc. cit.*) :

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

where u is the electrophoretic mobility, Q is the charge on the particle, l and a are respectively the length and the radius of the cylindrical colloid particle, r_i is the average radius of the ions in the ionic atmosphere, κ is the Debye–Hückel ionic strength function, $\mathrm{F}'(\kappa a)$ is the function developed by Gorin 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 which together with values of $\mathrm{F}'(\kappa a)$ have been given by Abramson, Moyer, and Gorin (*loc. cit.*).

The values of the electrophoretic mobility calculated from this equation are given in Table II, together with the observed values. For these calculations the values of l and a were taken as 2538 A. and 10·6 A. respectively; these values correspond to a value for the axial ratio of 120 and a molecular weight of $8{\cdot}2 \times 10^5$ (Cecil and Ogston, *loc. cit.*) and a specific volume of 0·55 c.c. per g. The particle charge data used in the calculations were taken from a smooth graph obtained from the data given in Table I. The value of r_i was taken as 2·24 A., this being the mean of the values for the sodium and chloride ions (Gorin, *J. Chem. Physics*, 1939, **7**, 405). The value of κ at 0·5° is $0{\cdot}323 \times 10^8\sqrt{I}$, and the viscosity of water at this temperature is $1{\cdot}76 \times 10^{-2}$ poise.

TABLE II.

Calculated and observed values of the electrophoretic mobility of the deoxypentose nucleate ion at pH 7 in solution of sodium chloride.

Ionic strength (I).	$\sqrt{I}$.	$\kappa(a + r_i)$.	$\mathrm{F}'(\kappa a)$.	$\frac{K_0(\kappa a + \kappa r_i)}{K_1(\kappa a + \kappa r_i)}$.	Q.	Mobility, calc.*	Mobility, obs.*
0·005	0·0707	0·293	6·53	0·440	550	3·21	3·20
0·01	0·100	0·414	6·48	0·515	565	2·81	2·79
0·02	0·141	0·584	6·44	0·591	595	2·49	2·48
0·05	0·224	0·913	6·31	0·692	690	2·31	2·02
0·08	0·283	1·17	6·10	0·738	780	2·37	1·83
0·10	0·316	1·31	5·97	0·754	830	2·41	1·74
0·20	0·447	1·85	5·72	0·800	1000	2·46	1·43

* Mobility given in μ/sec./(volt/cm.).

Discussion—(i) *Membrane potentials and particle charge.* The sources of error involved in the determination of membrane potentials have been described in detail by Adair and Adair (1934, *loc. cit.*), and as due consideration was given to their conclusions it is thought to be unlikely that in this investigation the experimental errors were greater than those accounted for by them.

The variation of the particle charge with ionic strength (Table I) is very great and, moreover, at all ionic strengths studied the charge is much lower than the value of 2,664 calculated from the analytical data assuming that the formula of the polynucleotide ion is

$$[(C_{39}H_{45}O_{24}N_{15}P_4)^{4-}]_{666},$$

which corresponds to the molecular weight of $8{\cdot}2 \times 10^5$, that the primary phosphoric acid groups are completely dissociated, and that the amino-groups are in the uncharged form at pH 7.

This variation of the particle charge from the theoretical value may be compared with the phenomena observed with Congo-red (Donnan and Harris, *J.*, 1911, **99**, 1554; Adair and Adair, 1934, *loc. cit.*) and with gum arabic (Svensson, *Arkiv Kemi, Min. Geol.*, 1946, **22**, *A*, No. 10). In both cases the equivalent concentration of the colloid as determined by membrane-equilibrium measurements was approximately half that calculated on the assumption of complete dissociation. The discrepancy in the case of the deoxypentose nucleate ion is considered to be due to the enmeshing of some of the sodium ions within the colloid micelle, thus reducing the apparent equivalent concentration of the sodium ions on which the membrane potential is dependent. The actual mechanism of this process is most probably ion-pair formation (see Svensson, *loc. cit.*) between sodium ions and phosphoric acid groups of the polynucleotide. Such sodium ions being bound within the surface of shear of the particle will not form part of the outer diffuse layer and will thus reduce the net charge on the particle.

The discrepancy between the observed and calculated values of the particle charge might be considered at first sight to be related to the anomalous osmotic properties of the deoxypentose nucleate ion reported by Hammarsten (*Biochem. Z.*, 1924, **144**, 383), who found that the osmotic pressures of solutions of this substance were lower than the values expected from electrical conductivity measurements. This " Hammarsten effect " was originally ascribed to the enmeshing of some of the sodium ions by the colloidal micelle thus rendering them osmotically inactive. Although it seems certain, from the data reported above, that this process does occur, it cannot explain the discrepancy between the osmotic pressure and conductivity data since the process of ion-pair formation would unavoidably lead to the prevention of such bound sodium ions taking part in the conduction process. The discrepancy between expected and observed osmotic pressures is therefore much more likely to be due to the great deviations from ideal behaviour which must be expected to occur with solutions of such interacting particles as deoxypentose nucleate ions (Linderström-Lang, *Compt. rend. Trav. Lab. Carlsberg*, 1926, **16**, No. 16; van Rysselberghe, *J. Physical Chem.*, 1934, **38**, 645), especially in the absence of a swamping concentration of electrolyte (Hartley, *Quart. Reviews*, 1948, **2**, 152).

The very great increase of particle charge with ionic strength cannot be ascribed solely to a variation in the extent of ion-pair formation, and is probably due to an increase in the adsorption of anions (either chloride or phosphate) by the deoxypentose nucleate ion as the ionic strength increases. This explanation may be compared with that put forward by Adair and Adair (*Biochem. J.*, 1934, **28**, 1230) to account for the behaviour of certain proteins.

(ii) *The calculation of electrophoretic mobility.* It is evident from the results given in Table II that there is close agreement between the observed and calculated mobilities at the lowest ionic strengths studied, but that discrepancies occur as the ionic strength is increased. While these discrepancies may in part be due to errors in the values of the particle charge incurred by the very small magnitude of the membrane potentials in this region, it is necessary to consider other factors that might possibly contribute to them. First, the actual charge effective in determining the mobility may not be identical with that given by membrane measurements. This explanation is considered to be unlikely since an ion which is adsorbed on the micelle sufficiently closely not to form part of the outer diffuse layer when the latter is undisturbed by the presence of an electric field would not normally be expected to become free when the field is applied (Svensson, *loc. cit.*).

Secondly, it is possible that at the higher ionic strengths considered, the charge on the particle is such that there are significant deviations from the assumption, made in the Debye–Hückel theory, that the electrical energy of the ions of the ionic atmosphere is small compared with their thermal energy. It is generally stated that the electrical energy term to be considered is $e\zeta$ (see, *e.g.*, Gorin, *loc. cit.*), where e is the electronic charge and ζ is the electrokinetic potential. However, the Debye–Hückel theory states that, for a sphere, the radial divergence of the potential gradient at any point in the ionic atmosphere is directly proportional to the potential at that point, provided that this potential is such that the electrical energy of an ion *at that point* is small compared with its thermal energy. Thus the energy term $e\zeta$ will be greater than the energy of most ions in the atmosphere, as relatively few will be in contact with the surface of shear, and, moreover, at high ionic strengths the potential falls sharply with the distance from the surface. Thus it is not possible to state any given value of the electrokinetic potential above which the Debye–Hückel theory does not apply, and tests of the Debye–Hückel theory generally consist of a direct comparison of the calculated and experimental values as carried out above. For spherical particles, Gorin (1941, *loc. cit.*; 1942, *loc. cit.*; private communication) has developed the Gronwall, La Mer, and Sandved

(*Physikal. Z.*, 1928, **29**, 358) extension of the Debye–Hückel theory; the resulting expression for the electrophoretic mobility can be used for particles of high charge, and gives mobilities lower in value than those given by the Debye–Hückel theory. A similar expression, applicable to cylindrical particles, has yet to be developed. It is thus concluded that present electrokinetic theory is inadequate to explain the mobility of cylindrical particles in solutions of ionic strength greater than about 0·05.

Experimental.

The determination of electrophoretic mobility has been described by Creeth, Jordan, and Gulland (Part IV, *loc. cit.*).

The membranes for the membrane-potential measurements were made by coating the inside of a boiling tube with two coats of a pyroxylin solution made according to the directions of Adair and Adair (*loc. cit.*), but were somewhat more porous than those described by these authors. In all other respects the apparatus employed was identical with that described by them except that normal calomel electrodes were employed and that in some measurements silver–silver chloride electrodes were used.

The solutions were prepared by dissolving a known weight of the tetrasodium salt of deoxypentose nucleic acid (previously dried in a vacuum at 100° over phosphoric oxide) in the appropriate buffer solution or solution of sodium chloride. The outer solutions, which did not contain the nucleic acid, were replaced by fresh solutions at intervals until further replacement produced no alteration in the membrane potential. The membrane potentials were measured on a potentiometer reading to 70 mv., graduated in steps of 0·02 mv. used in conjunction with a mirror galvanometer having a full scale deflection of 2 mv. All measurements were made at 0·5°.

The procedure for the determination of the membrane potential was as follows. The difference in potential of the two reference electrodes was first measured by placing them in opposition; two measurements of the membrane potential were then made followed by a repeat of the whole procedure with the two electrodes exchanged. The steady reading of the potential obtained 5 minutes after forming the liquid junction was taken as the observed membrane potential (Maclagan, *Biochem. J.*, 1929, **23**, 309).

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