299. *Deoxypentose Nucleic Acids. Part IV. The Electrophoresis of the Deoxypentose Nucleic Acid of Calf Thymus.*

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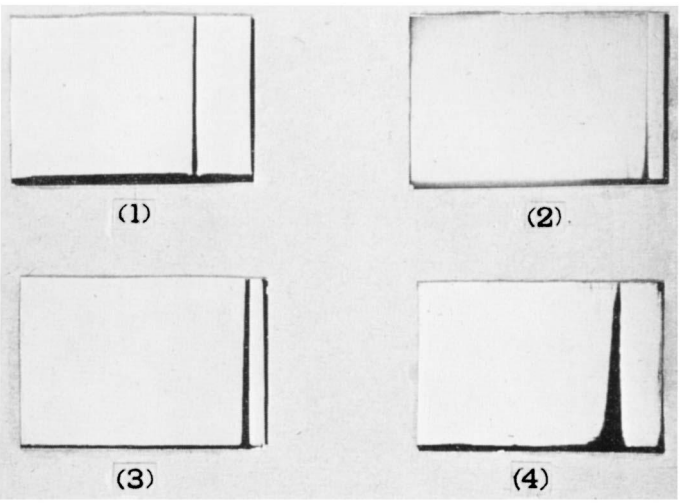
The electrophoresis of the sample of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus prepared by Gulland, Jordan, and Threlfall (*J.*, 1947, 1129) has been studied in the Tiselius apparatus. The variation of the mobility with changes in concentration, ionic strength, and pH have been investigated. The results, which are in agreement with and extend those of previous workers, are interpreted in terms of the macromolecular structure of this nucleic acid (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131; Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141).

The results of previous investigations of the electrophoretic mobility of deoxypentose nucleic acids (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) have shown that the deoxypentose nucleic acid of calf thymus is electrophoretically homogeneous with a high mobility towards the anode. The various recorded values of the mobility, however, show some discrepancy which can be attributed only in part to the different ionic strengths employed in these investigations. These variations have been ascribed by Cohen (*loc. cit.*) to a difference in the number of phosphoric acid dissociations per molecule in the various samples studied; furthermore it has been demonstrated that the molecular weight is dependent on the method of extraction (Schmidt, Pickels, and Levene, *J. Biol. Chem.*, 1939, **127**, 251; Tennent and Vilbrandt, *J. Amer. Chem. Soc.*, 1943, **65**, 424), and that treatment with acid or alkali produces irreversible changes in the macromolecular structure (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131; Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141). It was therefore considered desirable that the sample of the sodium salt of the deoxypentose nucleic acid of calf thymus which had been prepared and analytically characterised by Gulland, Jordan, and Threlfall (Part I, *J.*, 1947, 1129) and shown by electrometric titration (Gulland, Jordan, and Taylor, *loc. cit.*) and viscosity measurements (Creeth, Gulland, and Jordan, *loc. cit.*) to be little, if at all, degraded, should be subjected to an electrophoretic study.

Results of the Present Investigation.—In conformity with the results of previous investigators, this sample of deoxypentose nucleic acid was found to be electrophoretically homogeneous under all the conditions of ionic strength and pH studied (Fig. 1). In agreement with expectation from the known high molecular weight and monodisperse character of this material (Cecil and Ogston, *J.*, 1948, 1382), diffusion occurred slowly, and the boundaries remained sharp during the course of electrophoresis.

(i) *The variation of mobility with concentration of deoxypentose nucleic acid.* Stenhagen and Teorell (*loc. cit.*) observed that the mobility of a sample of the sodium salt of deoxypentose nucleic acid prepared by the method of Bang (Hofmeister's "Beiträge chem. Physiol. Path.," 1903, **4**, 331) as modified by Hammarsten (*Biochem. Z.*, 1924, **144**, 383) was independent of the concentration over the concentration range 0·05—0·25%, although the viscosity of the solutions was more than doubled over this range. Our results, which were obtained at constant ionic strength (0·20 due entirely to NaCl) are given in the table, and show only a very slight increase of mobility with concentration although the viscosity has increased by more than tenfold. This result thus confirms the conclusion of Stenhagen and Teorell (*loc. cit.*) that a viscosity correction should not be applied to these measurements.

FIG. 1.



Electrophoretic patterns of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus. Concentration 0·2%, *ionic strength* 0·2. *The ascending boundary (migration towards the anode) is shown in each case:* (1) *after* 12×10^3 *secs. at* 1·42 *volt/cm., phosphate–chloride buffer, pH* 7·0; (2) *after* $6{\cdot}12 \times 10^3$ *secs. at* 2·16 *volt/cm., phosphate buffer, pH* 11·7; (3) *after* $10{\cdot}38 \times 10^3$ *secs. at* 2·90 *volt/cm., acetate–chloride buffer, pH* 3·57; (4) *after* $13{\cdot}92 \times 10^3$ *secs. at* 1·57 *volt/cm., phosphate–chloride buffer, pH* 6·70, *after alkaline treatment at pH* 12·0.

[*To face p.* 1406.

Concn. of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus (%).	Electrophoretic mobility towards anode.[1]	Relative viscosity [2] at an applied pressure of 8000 dynes/cm.[2].
0·05	1·39	3
0·10	1·44	7
0·20	1·43	21
0·30	1·50	40

[1] These and all subsequent values of mobilities are stated in μ/sec./(volt/cm.).
[2] Values of the viscosity were determined by the method of Creeth, Gulland, and Jordan (*loc. cit.*).

(ii) *Dependence of the mobility on the ionic strength.* The ionic strength was varied by using sodium chloride as added electrolyte. The absence of buffering capacity of the deoxypentose nucleate ion at pH 7·0 (Gulland, Jordan, and Taylor, *loc. cit.*) renders the mobility change across the boundary negligible, and the migration is free from boundary anomalies. The results obtained are shown in Fig. 2, and it is evident that the mobility is greatly influenced by the ionic strength. There is a close similarity between the variation of mobility and the variation of viscosity with ionic strength (Creeth, Gulland, and Jordan, *loc. cit.*) which is in accordance with the suggestion that the change of viscosity with ionic strength is due to changes in the structure of the ionic atmosphere (Gulland and Jordan, *Symp. Soc. Exp. Biol.*, 1947, **1**, 56). The interpretation of these data with reference to the theory of electrophoretic migration is discussed in Part V (following paper).

FIG. 2.

Variation of the electrophoretic mobility of the deoxypentose nucleate ion with ionic strength in solutions of sodium chloride.

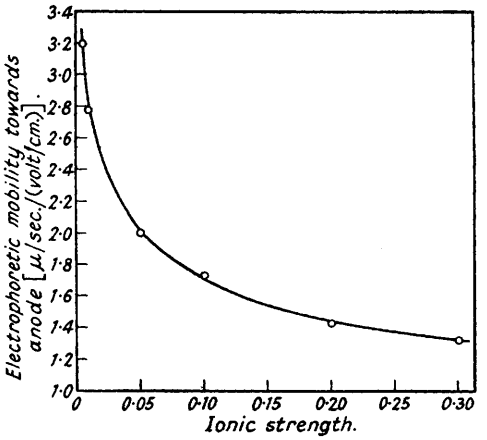
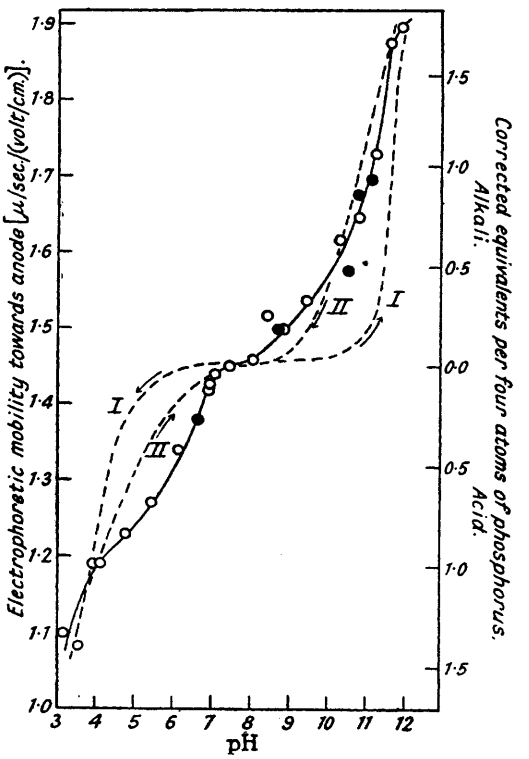


FIG. 3.

Variation of the electrophoretic mobility of the deoxypentose nucleate ion with pH. Ionic strength 0·20.



○ *Solutions brought directly to the given pH;* ● *solutions brought to the given pH after treatment with alkali at pH 12·0. Broken curve I, forward titration with acid or alkali from pH 7·0; broken curve II, backward titration with acid from pH 12·0 or with alkali from pH 2·5.*

(iii) *The variation of mobility with pH.* Stenhagen and Teorell (*loc. cit.*) investigated the acid–base binding and mobility–pH curves for the sample of deoxypentose nucleic acid studied by them, and reported a general similarity between the two. Their measurements, however, were restricted to the pH range 2·5—10·0, and, in view of the change in macromolecular structure occurring at pH values greater than 10·0 and the subsequent titration of the purine-pyrimidine hydroxyl groups between pH 10·0 and 12·0, it was expected that the mobility changes over this pH range would prove of interest; measurements of the mobility have therefore been made over the range pH 3·0—12·0.

The buffer solutions used were acetate, phosphate, borate, veronal, citrate, and carbonate, and in general the buffer contributed to not more than half the final ionic strength (0·20), the remainder being sodium chloride. By this means and by avoiding the use of potassium salts (Longsworth and MacInnes, *Chem. Reviews*, 1939, **24**, 271; Longsworth, *Ann. N. Y. Acad. Sci.*, 1941, **41**, 267) specific effects due to individual buffer ions were minimised. However, citrate buffers were found to give abnormally high mobilities of the deoxypentose nucleate ion, thus resembling the behaviour of phycoerythrin in these buffers reported by Tiselius (*Nova Acta Reg. Soc. Sci. Upsaliensis*, 1930, **1**, No. 4), and borate buffers were found to give rather low mobilities in agreement with the observations of Stenhagen and Teorell (*loc. cit.*). In the latter case the anomalous behaviour disappeared as the borate concentration was reduced at constant ionic strength. The mobility values in veronal buffers, which are recommended by Longsworth, Shedlovsky, and MacInnes (*J. Exp. Med.*, 1939, **70**, 399) for electrophoresis measurements, were almost identical with those obtained in phosphate buffers.

A representative series of photographs of the boundaries after electrophoresis under various conditions is shown in Fig. 1, and the mobility–pH curve obtained, together with the acid–base binding curve, is given in Fig. 3. The latter curve, taken from the data of Gulland, Jordan, and Taylor (*loc. cit.*), has been adjusted to the same scale as the mobility–pH curve by a method similar to that described by Abramson, Moyer, and Gorin (" The Electrophoresis of Proteins," Reinhold Publishing Corporation, 1942). It is evident that there is a close similarity between the two curves, but it is noteworthy that the back-titration curve, rather than the forward-titration curve, is followed. Experiments undertaken to construct a curve analogous to the back-titration curve, by treating a sample of the deoxypentose nucleic acid with alkali at pH 12·0 and then reducing the pH to the desired value and adjusting the ionic strength before determining the mobility, gave a series of points on the original mobility curve (see Fig. 3), thus enhancing the resemblance of the latter to the back-titration curve.

Discussion.—The homogeneity of electrophoretic migration shown by this sample of deoxypentose nucleic acid under all the conditions studied, when considered in conjunction with the sedimentation and diffusion data of Cecil and Ogston (*loc. cit.*), is strong evidence for a high degree of molecular homogeneity. The constancy of the mobility with increasing concentration of the deoxypentose nucleate ion and resulting increase of viscosity of the solution indicates that the viscous retarding force acting on the particles is determined by the viscosity of the solvent. This conclusion is in conformity with the theoretical prediction that, at the high ionic strength employed, the thickness of the double layer of the colloidal ion is small, and therefore only a statistically insignificant proportion of the colloidal solute can be included within the double layer (cf. Abramson, Moyer, and Gorin, *loc. cit.*).

The similarity of the mobility–pH and electrometric titration curves (Fig. 3) indicates that the electrophoretic mobility of the deoxypentose nucleate ion is largely dependent on the acid–base characteristics. The fall in mobility as the pH is decreased from neutrality was ascribed by Stenhagen and Teorell (*loc. cit.*) to the decreased ionisation of the primary phosphoric acid groups. In view of the conclusion of Gulland, Jordan, and Taylor (*loc. cit.*) concerning the nature of the groups undergoing titration in this range this view is no longer tenable, and it is now attributed to the ionisation of the amino-groups causing a decrease in the net negative charge.

The small increase in mobility over the pH range 7·0—10·0 is in agreement with the absence of buffering power over this range (Gulland, Jordan, and Taylor, *loc. cit.*) when that part of the charge on the particle due to ionisation is sensibly constant. The sharp increase in the slope of the curve in the region of pH 11·0, and the maximum value of the mobility attained at pH 12·0, are consistent with the view, suggested by the electrometric titration and viscosity data, that there is a sudden liberation of dissociating groups from an initially hydrogen bonded aggregate.

In view of the absence of any difference between the mobilities of the normal and alkali-treated samples of this substance in the region of pH 10·0, similar to the discrepancy in this region of the forward- and back-titration curves (Gulland, Jordan, and Taylor, *loc. cit.*), it must be concluded that the factors which determine the mobility of the less asymmetric particles considered to be produced by this change are similar to those previously operative on the original aggregates. The further interpretation of the mobility data in this pH region and also at pH 5 must await a more complete knowledge of the size, shape, and charge of the deoxypentose nucleate ion.

Experimental.

Solutions were prepared by dissolving in water, with stirring, a known weight of the sodium salt of deoxypentose nucleic acid, previously dried in a vacuum at 100° over phosphoric oxide, to give a

solution of twice the desired concentration. This solution was then diluted with an equal volume of buffer solution of twice the desired ionic strength. By adopting this procedure, the need for prolonged dialysis was avoided. This was considered necessary in view of the changes in macromolecular structure which result from prolonged contact with acid or alkaline solutions.

The mobilities were determined at 0·5° in a modified form of the electrophoresis apparatus (constructed by Adam Hilger Ltd.) described by Tiselius (*Trans. Faraday Soc.*, 1937, **33**, 524) and equipped with the cylindrical lens and diagonal edge form of optical system introduced by Philpot (*Nature*, 1938, **141**, 283). The conditions necessary for the accurate determination of mobilities with this apparatus have been described by Tiselius (*loc. cit.*), Longsworth and MacInnes (*J. Amer. Chem. Soc.*, 1940, **62**, 705), and Svensson (*Arkiv Kemi, Min. Geol.*, 1946, **22**, *A*, No. 10). A colloid concentration of not more than 0·20% was used, and where possible buffer solutions of high ionic strength were employed. Under these conditions the specific conductivities of the deoxypentose nucleic acid solutions and of the buffer solutions were sensibly identical, and boundary anomalies almost completely depressed.

The movements of the boundaries were recorded photographically, four positions of the boundary generally being obtained during an experiment over a period of 3—4 hours. During this time the movement of the boundary was of the order of 4 cm. Measurement of the distances on the photographic plate was made with a travelling microscope reading to 0·002 cm. The motions of both the ascending and descending boundaries were in general identical within the limits of error of measurement, and mobilities were therefore generally calculated from the ascending boundaries which were somewhat sharper. Mobilities were calculated from the formula $u = \Delta x.q.k_s/i.\Delta t$ where Δx is the distance travelled by the boundary in time Δt, q is the cross-sectional area of the U-tube limb, k_s is the specific conductivity, and i the current flowing. The mobility values were not corrected for variation of the viscosity of the different buffer solutions employed in view of the uncertain significance of this correction (Svensson, *loc. cit.*). The pH values were measured by the method of Jordan and Taylor (*J.*, 1946, 994), and conductivities were determined by the use of a Mullard resistance bridge. Both measurements were made at 0·5°.

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