[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Physical-chemical Investigation of Certain Nucleoproteins. II. General Electrophoretic Behavior

BY JAMES L. HALL[1,2]

Introduction.—The effort is being made in this Laboratory to begin physico-chemical studies on an important class of proteins which have not thus far been adequately investigated. In a previous report Carter and Hall[3] described the preparation and some general properties of a nucleohistone of the calf thymus and a nucleoprotein of the hog thyroid. In spite of the fact that comparatively little is known concerning the chemical nature of these proteins, we have succeeded in making available information about their electrokinetic and molecular kinetic behavior. This information will, among other things, aid in an eventual complete chemical characterization. In this report we consider the electrophoresis, as influenced by acidity and the presence of electrolytes, not only of the nucleoproteins but also of the histone and the nucleic acid of the calf thymus. In another article to follow, their molecular-kinetic properties will be described.

Method and Description of Apparatus.—The moving boundary electrophoretic method was used in this investigation. A portion of the experiments were performed in an apparatus similar to that described by Tiselius[4] in which use was made of the Lamm[5] scale method for determination of the position and concentration gradient of the boundaries. The U-tube of the assembly was immersed in a thermostat at 25°. The newer Tiselius equipment, first described in 1937,[6] was used for the remainder of the experimental work. The position of the boundaries was then determined by either the Lamm scale method or by visual observation of the schlieren bands on a ground glass plate. These experiments were performed at 0.7°.

Calculation of Mobilities.—The mobilities were calculated by means of the formula, $u = \frac{\Delta x}{\Delta t} \cdot \frac{q\kappa}{i}$, where u is the calculated mobility, Δx is the distance moved in centimeters in time, Δt, q is the cross sectional area of the U-tube in square centimeters, i is the current in amperes, and κ is the specific conductance in mhos of the buffer solution. In the case of data obtained by use of the Lamm scale method, the position of the boundary at any instant was taken as the ordinate through the point on the integrated form of the line displacement curve at abscissa 0.5. When schlieren observations were made, the mid-point of the band was used as the boundary position.

Mobility data presented in the tables and graphs are approximations of the limiting mobility at zero protein concentration which have been computed by taking an average of the mobilities computed from the velocity of the rising boundary, u_r, and from the velocity of the descending boundary, u_d. Longsworth and MacInnes[7] show for egg albumin that mobilities calculated from the velocity of the descending boundary "change but slightly with protein concentration and much of this change disappears if the mobilities are multiplied by the relative viscosity." Our work has demonstrated important differences between the moving boundary electrophoretic behavior of the nucleoproteins and of egg albumin, and it is especially noticeable that the mobility calculated from the velocity of the downward boundary gives a value which varies greatly with protein concentration, even when multiplied by the relative viscosity of the solution. The reason for the decision to use the average mobility will be apparent from a study of Fig. 1. As the concentration of the nucleohistone is increased, the velocity of the rising boundary increases at first, but then it falls off somewhat, while the rate of movement of the descending boundary decreases sharply and steadily. The form and position of these curves are doubtless largely determined by the high viscosity of the solutions (elongated particle shape[3]). From the figure it is seen that in the protein concentration range up to 0.2 to 0.3%, the average of the mobilities, $(u_r + u_d)/2$, gives

(1) More complete details of this work may be found in a thesis by James L. Hall, presented in June 1939 to the Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Original manuscript received February 17, 1940.

(2) Present address: Department of Chemistry, Tufts College, Medford, Mass.

(3) Carter and Hall, THIS JOURNAL, **62**, 1194 (1940).

(4) Tiselius, Dissertation, Upsala, 1930.

(5) Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); **A143**, 177 (1929).

(6) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(7) Longsworth and MacInnes, THIS JOURNAL, **62**, 705 (1940).

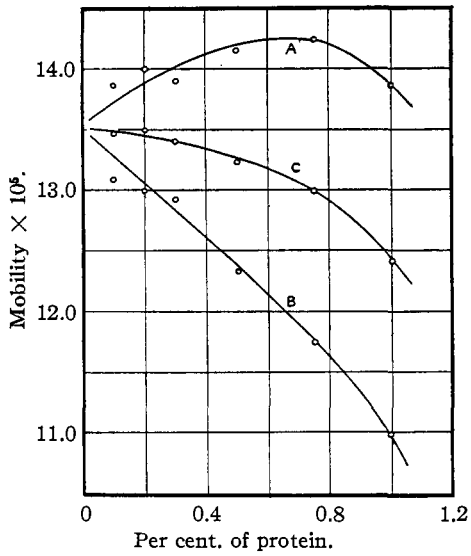

Fig. 1.—Concentration–mobility data for thymus nucleohistone in phosphate buffers of *p*H 6.97 and ionic strength 0.02: (A) mobility calculated from the rate of the rising boundary; (B) mobility calculated from the rate of the descending boundary; (C) average of (A) and (B).

a better estimate for the limiting mobility than does either the rising or the descending value alone. It was in this concentration range that most of our experiments were performed. The curves of Fig. 1 indicate the necessity for recording observations of the migrations of both boundaries when experiments are made with more concentrated protein solutions.[8]

Preparation of Materials.—The preparation of the calf thymus nucleohistone and of the hog thyroid nucleoprotein have been described previously.[3] Additional nucleoproteins of the calf thymus were prepared by the method of Huiskamp.[9] These proteins were precipitated by dilute acid after removal of the nucleohistone. Two fractions of this material were distinguished by their solubility. One is easily soluble in a dilute buffer solution at *p*H 8 while the other is dissolved only upon the addition of ammonia. These will be referred to as "water soluble" and "ammonia soluble" nucleoprotein fractions.

The histone and the nucleic acid were prepared from the calf thymus nucleohistone. The histone was isolated by the method of Bang[10] while the nucleic acid was obtained as the sodium salt following the procedure of Hammarsten.[11]

(8) *Cf.* Davis and Cohn, THIS JOURNAL, **61**, 2092 (1939).
(9) Huiskamp, *Z. physiol. Chem.*, **32**, 145 (1901).
(10) Bang, *Beitrag chem. Phys. Pathol.*, **1**, 189 (1902); **4**, 115, 362 (1903); **5**, 317 (1904).
(11) Hammarsten, *Biochem. Z.*, **144**, 383 (1924).

Only freshly prepared materials were used in all determinations. Electrophoresis experiments were preceded by dialysis in Visking tubes to equilibrium with the buffer solution.

Solubility Behavior.—The calf thymus nucleohistone is precipitated by 0.1% calcium chloride or 1.0% sodium chloride and is readily redissolved in 1 and 5% solutions of these reagents, respectively. It is insoluble in dilute buffer solutions below *p*H 5. The hog thyroid nucleoprotein and the calf thymus water soluble and ammonia soluble fractions do not dissolve in dilute buffers from *p*H 3.5 to *p*H 5. The histone is insoluble in a limited region near *p*H 12.

Electrophoretic Data. Temperature 25°.—In electrophoresis the calf thymus nucleohistone behaves as a single homogeneous substance. The experiments were performed in a variety of buffer solutions from *p*H 5 to *p*H 8. Figure 2 shows the protein concentration gradient–cell position distance curve, determined by the Lamm scale method, at the limit of migration in buffer solution at *p*H 7.72. All other experiments in

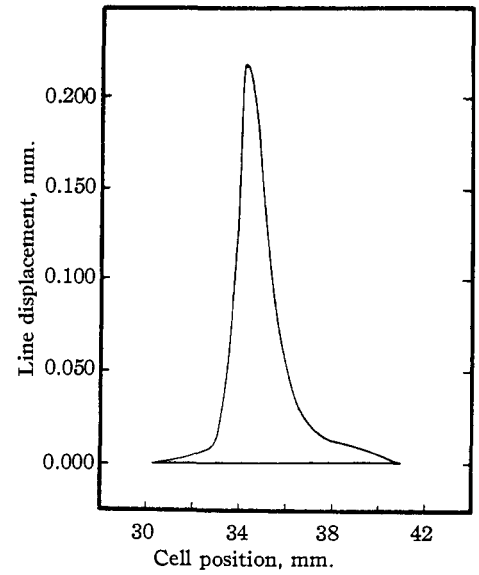

Fig. 2.—Concentration gradient–cell position curve for thymus nucleohistone at the limit of migration in an experiment buffered at *p*H 7.72: protein concentration 0.30%.

solutions buffered from *p*H 5 to *p*H 8 gave similar results. The mobility data are summarized in Fig. 3. The experiments were carried out with 0.2 to 0.3% protein solutions. The mobilities are average values calculated as previously described. Determinations of *p*H made with the

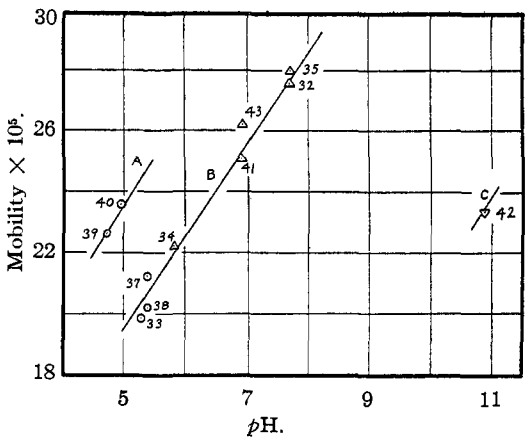

Fig. 3.—*p*H–mobility data for calf thymus nucleohistone at 25.0°: (A) ionic strength 0.10; (B) ionic strength 0.02; (C) ionic strength 0.10; ⊙, experiments in acetate buffers; △, experiments in phosphate buffers; ▽, experiment in borate–NaOH buffer. Protein for experiments 32, 33, and 38 precipitated once by NaCl; protein for experiments 34, 35 and 37 precipitated once by $CaCl_2$; protein for experiments 39, 40, 41, 42, and 43 precipitated twice by NaCl.

dialyzed protein and buffer solutions showed only negligible differences (less than 0.02 *p*H unit). The electrophoretic behavior of the nucleohistone is independent of the mode of preparation (calcium chloride or sodium chloride precipitation).

In buffer at *p*H 10.90 the nucleohistone reveals some electrical inhomogeneity as is shown by Fig. 4. When nucleohistone is dialyzed against 0.1 *N* sodium hydroxide for one week, then returned to *p*H 6.95 for electrophoresis, about 90% of the protein present has an electrophoretic behavior identical with that of untreated nucleohistone. The remaining portion migrates at a slower rate and is very inhomogeneous.

Two series of experiments were carried out with different preparations of the nucleoprotein of the hog thyroid.[3] This protein was studied on both

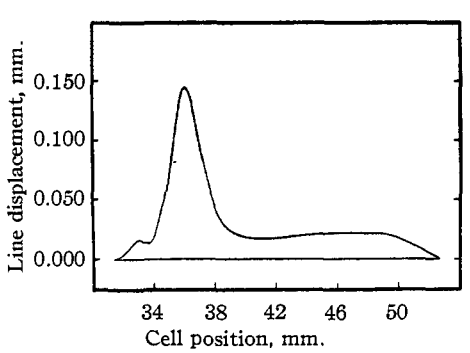

Fig. 4.—Concentration gradient–cell position curve for thymus nucleohistone at the limit of migration in buffer at *p*H 10.90; protein concentration 0.30%.

sides of its isoelectric insoluble region. In its electrophoretic behavior it appears to be homogeneous. Repeated precipitation by either acetic or hydrochloric acid yields a product with the same electrical properties. In all cases the boundaries give concentration gradient–distance curves which are similar to the ones shown in Fig. 2.

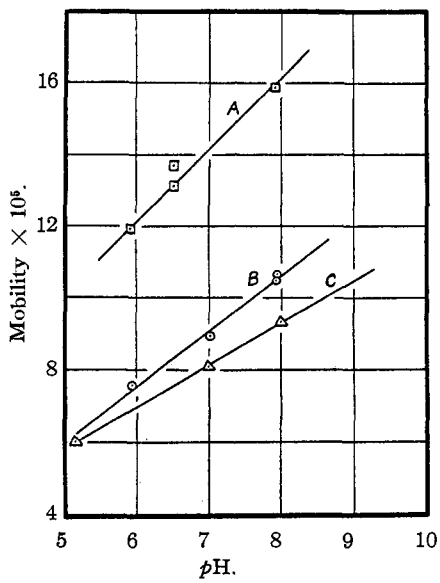

Fig. 5.—*p*H–mobility data at 0.7° for (A) calf thymus nucleohistone, (B) "water soluble" and (C) "ammonia soluble" nucleoprotein fractions of the calf thymus.

The clarified thymus gland extract was also subjected to electrophoresis. The protein in this solution is quite inhomogeneous. More rapidly moving components predominate in amount. No exact mobility determinations were attempted, although estimates indicate that the most rapidly moving component in the extract is migrating at a rate slower than that of the thymus nucleohistone prepared from the extract by sodium chloride precipitation. It would probably be very difficult to isolate distinct components from the gland extract by electrophoretic separation.

Electrophoretic Data. Temperature 0.7°.—The *p*H–mobility curve for the calf thymus nucleohistone was determined in the new Tiselius apparatus at the lower temperature. The data are plotted as a part of Fig. 5. Experiments with this protein and at this temperature were used to obtain the data from which Fig. 1 was prepared.

One experiment was performed with nucleohistone which had been precipitated by 1% sodium chloride and redissolved by dialysis against a phosphate buffer containing 4% sodium chloride.

Because of the very low potential which could be applied to this system several hours were required for an appreciable displacement of the boundaries. However, it was definitely established that the migration was anodic and therefore that the protein carries the same charge in concentrated salt solutions as in dilute buffer systems.

The ammonia soluble and the water soluble fractions of the calf thymus nucleoprotein were also studied. The ammonia soluble fraction behaves as one homogeneous component. The water soluble part yielded, in addition to the chief nucleoprotein constituent, a second fraction present in very small amount. Under corresponding conditions the mobility of this lesser fraction is much greater than that of the nucleoprotein and nucleohistone but less than the mobility of the nucleic acid derived from the nucleohistone. The data obtained for the ammonia soluble fraction and for the chief constituent of the water soluble fraction are presented graphically in Fig. 5. They establish these two proteins as being distinct from one another and from the nucleohistone already studied.

Four experiments were performed with the histone of the calf thymus. In phosphate buffer at a constant ionic strength of 0.02 the mobility is 5.9×10^{-5} at *p*H 6.98 and 4.9×10^{-5} at *p*H 7.84. In carbonate–borate buffer systems of the same ionic strength mobilities of 6.5×10^{-5} and 8.1×10^{-5} are obtained at *p*H 10.04 and 9.50, respectively. These migrations were cathodic in direction. The ionic strengths were calculated on a basis of complete ionization of the salts involved, and the great variation of this property between the two buffer systems probably accounts for the greater mobilities in the more alkaline carbonate–borate system.

The mobility data for sodium thymonucleate are presented in Table I. Although this material appears to be quite homogeneous, accurate mobility determinations were difficult because of the very high viscosity of the solution and rapid movement of the boundary. All experiments were carried out in 0.1% solution. These *p*H–mobility data agree well with values published, after completion of our experiments, by Stenhagen and Teorell,[12] if proper allowance is made for the different ionic strengths of the solutions used in the two laboratories.

TABLE I

MOBILITY DATA FOR SODIUM THYMONUCLEATE IN 0.1% SOLUTION AT 0.7°

Expt.	Buffer	Ionic str.	*p*H	Mobility cm.^{2} sec.$^{-1}$ volts^{-1} × 10^5
29	Phosphate	0.02	7.80	23.5
27	Phosphate	.02	7.01	21.0
28	Phosphate	.02	5.97	21.0
33	Acetate	.02	5.00	20.5
37	Phosphate–citrate	.03	3.92	19.2
34	Phosphate–citrate	.03	3.37	17.1

Discussion.—Because of the insolubility of the nucleohistone, the nucleoproteins, and the histone in their isoelectric regions, extrapolation of mobility data to obtain an apparent isoelectric point for each is impossible. It is recognized and was shown that *p*H–mobility curves are shifted as the ionic strength of the solution is changed. For calf thymus nucleohistone at ionic strength 0.02, our experiments locate the isoelectric point within the region *p*H 3.5 to 4.3. The isoelectric points of the hog thyroid nucleoprotein and the calf thymus nucleoproteins are also near *p*H 4. The isoelectric point of the histone lies near *p*H 12.

The nucleoproteins and the nucleohistone all appear to be quite stable with respect to dilute alkali in so far as electrophoretic behavior is concerned. The nucleoproteins are also unaffected by 0.1 *N* hydrochloric acid. There is nothing in the electrophoretic data to indicate a lack of stability from *p*H 3 to *p*H 9. All of these proteins retain the same electrical properties although preparational methods have been appreciably varied. This evidence supports the contention that the nucleoproteins are definite compounds with a constant ratio of nucleic acid to protein.

The changes in electrophoretic mobility of the nucleohistone between the two temperatures, 25 and 0.7°, are quantitatively accounted for by the difference in the viscosities of the buffer solutions caused by the temperature change.

Acknowledgment.—The writer wishes to thank J. W. Williams for his assistance, advice and encouragement with this research. The Wisconsin Alumni Research Foundation contributed funds for the purchase of part of the equipment used. Assistance in the installation of apparatus was furnished by the personnel of Work Projects Administration Official Project No. 65-1-53-2349.

Summary

The nucleohistone of the calf thymus, two

(12) Stenhagen and Teorell, *Trans. Faraday Soc.*, **35**, 743 (1939).

additional nucleoproteins of the calf thymus, and the nucleoprotein of the hog thyroid have been characterized as substances uniform in their electrophoretic behavior.

For this class of proteins, the isoelectric point is near *p*H 4. Exact determinations are impossible because of their limited solubility.

These proteins have much greater mobilities in the intervals studied, *p*H 2 to 3.5 and *p*H 5 to 9 than do the usual non-conjugated proteins.

The corresponding thymonucleic acid and histone have been prepared, and each is found to be electrophoretically homogeneous. The histone is insoluble at its isoelectric point which is in the vicinity of *p*H 12.

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Polymeric Methyl Silicon Oxides[1]

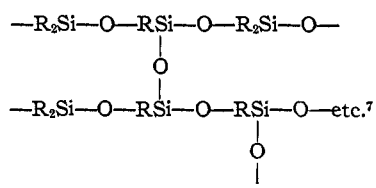
BY E. G. ROCHOW AND W. F. GILLIAM

While the hydrides and alkyls of tetravalent silicon are monomeric and volatile, their oxygen derivatives usually exist in the form of polymeric structures of alternate silicon and oxygen atoms.[2] Thus prosiloxane[3] has liquid and solid forms, and the silicones[4] are usually polymeric. Since these organic silicon-oxygen chains resemble those of silica and the inorganic silicates, we may properly speak of the compounds generally as organo-silicon oxides.

This paper is concerned with some new polymeric methyl silicon oxides which attain their polymeric structure by a process of condensation of the corresponding methyl silicols. Our interest in this field was aroused by Dr. J. F. Hyde of the Corning Glass Company, who showed Dr. A. L. Marshall of this Laboratory some samples of phenyl silicone which he considered promising as impregnants for glass fiber tape.

Kipping[5] first recognized that the silicanediols $R_2Si(OH)_2$ condense intermolecularly to yield polymeric anhydrides and silicones. The silicanetriols $RSi(OH)_3$ likewise were known to condense to white, powdery "siliconic acids" $(RSiOOH)_x$.[6]

During the course of an investigation of the phenyl and alkyl silicones in this Laboratory, it was found that the silicanediols may be made to condense intermolecularly with the silicanetriols to yield solid polymers, probably of a cross-linked siloxane structure:



Within a certain range of R/Si ratios, characteristic for each R group and decreasing as the size of the R group was increased, the polymers were found to be resinous solids of high thermal stability.

Since the interesting properties of such resins probably derive from the siloxane linkage, the greatest advantage of such a linkage was sought in a polymer having a minimum organic content and a maximum of silicon and oxygen. While methyl silicon oxide was the logical choice, the methyl silicon chlorides and methyl silicols to be used as starting materials were heretofore unknown. Preliminary attempts to isolate the methyl silicon chlorides were unsuccessful, but it was discovered that a methyl silicon oxide could be prepared by direct hydrolysis of the reaction products resulting from the action of methylmagnesium bromide on silicon tetrachloride in ether. When such a reaction mixture was poured on ice, hydrolysis of the chlorides and condensation of the resulting silicols occurred simultaneously, giving liquid products which could be condensed further to resinous solids. The subsequent iso-

(1) This paper was read on September 10, 1940, at the 100th Meeting of the American Chemical Society at Detroit, Michigan.

(2) Sidgwick, "The Electronic Theory of Valency," Oxford Univ. Press, New York, N. Y., 1932, p. 159.

(3) Stock, *Ber.*, **50**, 1769 (1917); **49**, 108 (1916).

(4) Kipping, *J. Chem. Soc.*, **101**, 2106 (1912); Krause and von Grosse, "Die Chemie der metallorganischen Verbindungen," Berlin, 1937, pp. 278–286.

(5) Kipping, *ibid.*, **91**, 218 (1907); **93**, 439 (1908); **95**, 302 (1909); **101**, 2106 (1912); **101**, 2142 (1912).

(6) Kipping, *ibid.*, **105**, 679 (1914); **107**, 459 (1915); Ladenburg, *Ann.*, **164**, 300 (1872); **173**, 147 (1874).

(7) A similar structure has been proposed by Andrianov and his coworkers for the film-forming products obtained by hydrolyzing alkyl-substituted silicon esters; see *Org. Chem. Ind. U. S. S. R.*, **6**, 203 (1939); *J. Gen. Chem. U. S. S. R.*, **8**, 552, 558, 1255 (1938); also Kalinin, *Compt. rend. Acad. Sci. U. S. S. R.*, **18**, 433 (1938). Andrianov does not report any work on the methyl-substituted esters.