SOME PHYSICO-CHEMICAL STUDIES ON NUCLEIC ACIDS AND RELATED SUBSTANCES.

Thesis, presented by James Michael Creeth, B.Sc., for the degree of Doctor of Philosophy in the University of London.

University College,
Nottingham,
1947.

ACKNOWLEDGEMENTS

My sincere thanks are due to the late Professor J.M.Gulland,F.R.S., for the continual encouragement and guidance he gave me up to the time of his death.

I wish also to express my gratitude to Dr.D.O.Jordan for his almost daily help and advice, and to all those with whom I was privileged to work at Nottingham; to Imperial Chemical Industries,Ltd., for the loan of apparatus, and to the British Empire Cancer Campaign for the grant which enabled the work to be carried out.

CONTENTS

CHAPTER I.

Introductory: The Structure and Isolation of Nucleic Acids.

Section (i) Introductory

(ii) The Deoxyribonucleic Acid of Calf Thymus Gland.

(iii) Isolation and Analysis of the Sodium Deoxyribonucleate used in the present investigation.

Section (i) Introductory.

The chemistry of the nucleic acids has been of outstanding interest to biochemists since the results of Miescher's investigations were published in 1871 (1). The unusual properties of the material he isolated from the bare nuclei of pus cells, and from salmon spermatozoa (also, in effect, a source of free nuclei) were later found to be common to materials extracted from many diverse plant and animal tissues. To this type of substance, characterised by its high phosphorus content, acidity, and density, he gave the name nuclein, a term which was modified to nucleic acid by Altmann (2) as more became known of the structure of these substances.

It became evident, as the result of many investigations, that nucleic acids were to be found in all living matter; indeed, the simplest organisms which show the essential characteristics of life, the plant viruses, consist of nucleoprotein alone. In addition, Caspersson (3) has shown that the chromosomes, the longitudinal splitting of which during mitosis is the basis of cell division, consist of nucleoprotein; it is apparent, therefore, that these nuclear substances must play an important, if not dominant, part in the phenomena of reproduction. Thus for an

understanding of the processes occurring in the growth of both healthy and malignant tissue, it is essential that the structure and physico-chemical properties of the nuclear material should be well defined.

The comparative ease with which nucleic acids could be prepared in quantity was a further incentive to their investigation by the degradative methods of organic chemistry, and many workers have helped to piece together the complex structure of the substances; among these investigators the names of Kossel, Steudel, Jones and Levene are pre-eminent.

Owing to the lack of understanding of the complex polymeric nature of the substances, many conclusions regarding their structure reached by early investigators proved to be unsound when submitted to rigid examination. The position has been reviewed by Gulland, Barker and Jordan (4); they examined the various uncertainties of the structure, and also pointed out the fact that results so far obtained can only have a statistical significance when applied to such complex long chain polymeric systems.

Certain of the earlier assumptions have accordingly been modified in the light of subsequent knowledge. Thus the conception of nucleic acids existing as tetranucleotides, which has grown up through the discovery of the four

nucleotides, in approximately equimolecular proportions, in the hydrolysate of yeast nucleic acid, and the fact that molecular weight determinations, in one case, indicated a particle size corresponding to a molecule composed of the four nucleotides (5), was later modified as the complex nature was realised. The tacit assumption that the native nucleic acid was built up of uniform structural tetranucleotides, however, has no sound experimental basis, as was pointed out by Gulland, Barker, and Jordan (4), but the conception has considerable use for the interpretation of analytical and titration data.

Two types of nucleic acid have for long been recognised, (6),distinguished, among other differences, by the presence of a pentose sugar component in the one, and a deoxypentose sugar in the other. These were thought, until comparatively recently, to be typical of plant, and animal, nucleic acids respectively; very often conclusions from investigations on the one were assumed to apply also to the other.

The work of Feulgen and others (7,8),however, indicated that the pentose containing acid is associated with the cytoplasm, and the deoxypentose acid with the nucleus, of the cells of living matter of both plant and animal origin. In certain cases, these sugars have been identified as d(-)ribose and d(-)2deoxyribose respectively, and it is usual to refer to these substances as ribo- and deoxyribo- nucleic acids. In the absence of evidence to prove that nucleic acids of

the same class are identical when derived from different sources, it is customary to specify the particular origin. Thus the nucleic acid of yeast which was regarded as a typical plant nucleic acid, is referred to as yeast ribonucleic acid; similarly, the typical 'animal nucleic acid', thymonucleic acid, is called thymus deoxyribonucleic acid.

It is now recognised (9) that native nucleic acids of both classes are high polymers, and that the earlier conflicting evidence of molecular size arose from a lack of appreciation of the effect on the macromolecules of the vigorous reagents employed in their extraction. It is indeed obvious that for physico-chemical investigations to have any bearing on the behaviour of the material in vivo, this should have been extracted in as simple and non-degradative a manner as possible, for, though a substance may be analytically stable to the reagents employed in its extraction, the possibility of the occurrence of fundamental changes in the macromolecule is not excluded; the facile denaturation of proteins without change in empirical formula provides such an example. In all cases, moreover, the greatest caution should be used in interpreting the results.

A method of extraction conforming to these conditions was developed by Hammarsten (10) for the deoxyribonucleic

acid of calf thymus gland, and the substance he prepared was manifestly different from that extracted by hot alkali, being white, fibrous, and forming a very viscous solution. The elementary analyses of the materials prepared by the two methods were very similar.

The physical properties of his material were so striking that Hammarsten suggested the predominant importance in nuclear metabolism of the physical characteristics of the nuclear material, a suggestion which has been the inspiration of many subsequent workers.

It was pointed out by Melville (11) and by Campbell and Johnson (12) that the molecular weight and shape of the colloidal particles are the most important properties in determining the behaviour of its solutions; of the many manifestations of these properties, the viscosity presents several advantages for investigation. The small quantities of material required, together with the ease and rapidity with which reproducible measurements may be obtained, compensate in some degree for the greater difficulty of interpretation compared with, for example, ultracentrifuge or diffusion measurements.

The investigation of the physico-chemical properties of a material similar to that prepared by Hammarsten forms the subject matter of this thesis; in particular, the viscosities and electrophoretic mobilities under various conditions,

have been studied in detail.

The results obtained, and the conclusions formed, will, it is hoped, help to bridge the gap between our knowledge of the chemical structure and the biological function of this most important nuclear substance.

Section (ii)

The Deoxyribonucleic Acid of Calf Thymus Gland.

Before discussing the physico-chemical investigations and their theoretical basis, the evidence for the present structure of this nucleic acid will be briefly summarised.

The methods employed by Miescher and his contemporaries for the isolation of 'animal nucleic acids' from various sources were often very laborious and gave low yields; however, in 1894, Kossel and Neumann (13) discovered that the thymus gland from calves was rich in 'nucleic acid', and this source was much used by later investigators.

Although Miescher (1) had considered unwise the use of alkalies, acids, or heat in extracting nucleic acids from tissues, the difficulty of extraction by methods not involving such treatment was so great that Neumann's modification (14) of the Altmann technique (15) became general. In this method, the minced tissue was heated with 3% sodium hydroxide solution, and the filtered extract treated with acetic acid, when the denatured proteins were precipated. After

filtration, the nucleic acid was precipitated either as the sodium salt (by adding alcohol after neutralisation) or as the free acid (by adding hydrochloric acid). Feulgen (16) introduced slight modifications to this method, but the principle of alkaline extraction remained standard.

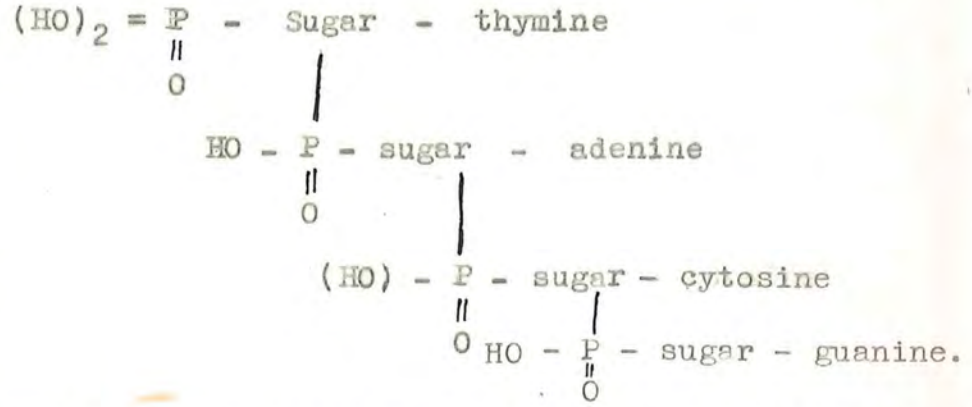
Evidence obtained from studies on materials prepared in this or a similar manner formed the basis for theories of the structure of deoxynucleic acid. The work of Levene (17), Jones and Austrian (18), Osborne and Harris (19), and Mandel (20) established the occurrence of the two purine bases, guanine and adenine, in equimolecular proportion; Kossel (21) Steudel (22) and Levene (23) showed that two pyrimidine bases, cytosine and thymine, were present; and the fact that all four nitrogenous bases were present in approximately equal proportions was demonstrated by the work of Steudel (24) and of Levene and Mandel (20).

The occurrence of a deoxypentose sugar was shown by Levene and London (25), and later, this was shown (26) to be d(-)2deoxyribose.

Electrometric titration data (Steudel (27), Levene and Simms (28)) showed the presence of four acidic dissociations per tetranucleotide. Later evidence (Feulgen (29) showed in addition the presence of two groups of $pK_a' \sim 10$, three

amino groups, and some secondary phosphoryl, the amount of this group varying somewhat in different samples.

On the basis of this, and other, mainly analytical, evidence, the structure of thymus deoxribonucleic acid was supposed by Levene and Simms (30) to be a straight sugar-phosphate chain compound of the four nucleotides:-



Other investigators (Makino (31), Bredereck and Köthnig (32) Stenhagen and Teorell (33) , Gulland, Jordan and Taylor (34) have studied the substance by this technique; the results obtained show some discrepancies which Cohen (35) has suggested may be ascribed to the different degrees of degradation of the various samples, a view which is supported by the evidence of Schmidt, Pickels and Levene (36) and of Tennent and Vilbrandt (37) from ultracentrifuge measurements.

The present day theory of the structure of thymus deoxyribonucleic acid is a modification of that proposed by Levene, in that the molecule is assumed to be highly

polymerised in a linear manner, no assumptions being made however, as to the order of the nitrogenous bases. Evidence for this conclusion which will be subsequently reviewed (ChapterIV) is based mainly on macromolecular studies on a material extracted by very mild methods. The importance of such non-degradative extractions has already been explained; the work of Hammarsten (38) based on a method devised by Bang (39) allowed the investigation, for the first time, of a material that approximated to its native condition. The subsequent results obtained by investigation of this material have been of great importance in a better understanding of nuclear processes, and will be fully discussed later.

The material used in the present investigation was prepared by a modification of the Hammarsten-Bang technique devised by Gulland, Jordan and Threlfall (40); the extraction was carried out by Mr. C.J. Threlfall, B.Sc.

Section (iii)

Isolation and analysis of the Sodium Deoxyribonucleate used in the present investigation.

The extraction process makes use of the remarkable solubility characteristics of the deoxyribonucleoprotein of the calf thymus gland. This substance is soluble in water, in dilute sodium chloride solutions (0.02M), and

in concentrated solutions (1 or 2 M), but is insoluble in physiological saline (0.14M). These properties have been adequately described by Mirsky and Pollister (41).

The finely minced tissue from fresh frozen glands is extracted twice with 0.9% (0.14M) sodium chloride solution by prolonged rapid stirring. The ribonucleoproteins and other cytoplasmic material dissolve, while the cytoskeleton and deoxynucleoproteins are left as an insoluble residue which is separated by centrifugation.

This tissue, which now gives negative tests for pentose, is extracted twice at 0°C with 10% sodium chloride solution, when the nucleoproteins dissolve and the solution becomes very viscous. After 48 hours, the cell debris is centrifuged off, and the nucleoprotein precipitated as a very fibrous white mass, by the addition of an equal volume of alcohol. The precipitate is washed with 70% alcohol until free from chloride, then with absolute alcohol, and finally with ether; it is then dried in vacuo. Yield, 1.69 Kg from 86.9 Kg of glands.

Mirsky and Pollister precipitated the nucleoproteins by making the salt concentration 0.9%. This procedure gives a precipitate which is less fibrous, and considerably more difficult to manipulate. The finely ground protein is then stirred rapidly with 10% sodium chloride solution,

to give an approximate 1% solution, and the insoluble matter (denatured protein) centrifuged off. The remainder of the protein is then removed by the method of Sevag, Lackmann and Smolens (42), whereby the salt solution is shaken repeatedly with a mixture of chloroform (1 part) and amyl alcohol (3 parts).

The precipitated protein forms a gel with the chloroform-amyl alcohol solution, and this is removed by centrifugation, the mixture separating into 3 layers: the top one being aqueous, the next, the gel of protein, and the bottom of excess chloroform and amyl alcohol. This process is repeated until no more gel forms at the interface, when the aqueous solution, which contains the sodium salt of the deoxyribonucleic acid, is no longer opalescent.

The combined aqueous layers are then mixed with an equal volume of alcohol, when the sodium deoxyribonucleate is precipitated as a white, very fibrous mass, which can be collected on a glass rod, as described by Mirsky and Pollister (41). It was washed with 70% alcohol until free from chloride, then with absolute alcohol, and finally with ether. The yield, after drying in vacuo, was 140 gm. from 500 gm. of nucleoprotein.

This procedure is probably preferable to the Hammarsten-Bang technique, whereby the protein is "salted-out" by saturating the aqueous solution with sodium chloride,

as the complete removal of the protein appears to be unlikely; in a sample prepared by this technique, Signer, Caspersson and Hammarsten (43) found traces of protein, and Gulland, Jordan and Taylor (34) have confirmed this result.

The product obtained by the method described above, however, gave negative biuret and Sakaguchi tests for protein. The significance of the method of extraction upon the macromolecular properties of the material isolated is a very interesting question and will be discussed later.

The elementary analyses of two samples of sodium deoxyribonucleate prepared in this way are given in the table.

Elementary Analytical Values for Sodium Deoxyribonucleate.

Sample	C	H	Purine N.	Pyrim.N.	Total N.	P.	Na
T5/1	35.4	3.6	9.4	5.9	15.3	9.33	7.1[1] 6.9[2]
T5/2	36.0	3.9	10.0	5.7	15.7	9.39	6.5[2]
Theoretical I	35.4	3.4	10.6	5.3	15.9	9.39	6.95
Theoretical II	35.4	3.4	10.0	5.6	15.6	9.41	6.97
Theoretical III	35.4	3.4	9.5	5.9	15.4	9.43	6.98

1. Analysis by the colorimetric method described by Hofmann and Osgood (44).
2. Analysis by the gravimetric method described in Chapter VIII.

The analytical values corresponding to "Theoretical I" are for the polymerised form of Levene tetranucleotide (p.8), containing one of each of the four nitrogenous radicles, of statistical formula $C_{39}H_{45}O_{24}N_{15}P_4Na_4$. It is evident that, with the exception of the values for the nitrogen content, the experimental figures for the sample T5/1 are in satisfactory agreement with the theoretical.

A possible explanation of this discrepancy has been suggested by Gulland, Jordan and Threlfall (40) from the results of a careful series of analyses of purine and pyrimidine nitrogen. They suggest that up to 20% of the adenine may have been replaced by cytosine; titration results (Gulland, Jordan and Taylor (34)) preclude the possibility of deviations from the theoretical in the case of guanine and of thymine. The values corresponding to Theoretical II and III, above, are calculated on the assumption that 10% and 20% respectively of the adenine has been replaced by cytosine.

The sodium and phosphorus analyses are in good agreement with the theoretical, indicating a base binding power of 4.0 equivalents of phosphoryl per tetranucleotide.

REFERENCES TO CHAPTER I.

1. Miescher, Hoppe-Seyler's Med. Chem. Untersuch. 1871, pp. 441, 502.
2. Altmann, Arch. Anat. u. Physiol., Physiol. Abt. 1889, p.524.
3. Caspersson, Skand. Arch. Physiol, Suppl. 1936, 73, No. 8.
4. Gulland, Barker and Jordan, Ann. Rev. Biochem, 1945, 14, 175
5. Myrbäck and Jorpes. Z. Physiol. Chem. 1935, 237, 159.
6. Kossel, Arch. Anat. u. Physiol, Physiol. Abt., 1891, p.181
7. Feulgen, Behrens and Mahdihassan, Z. Physiol. Chem, 1937, 246, 203.
8. Delaporte and Roukhelman, Compt. Rend. 1938, 253, 185.
9. Gulland, J.C.S. 1944, 208.
10. Hammarsten, Biochem. Z. 1924, 144, 383.
11. Melville, Trans. Farad. Soc. 1944, 40, 217.
12. Campbell and Johnson, Trans. Farad. Soc. 1944, 40, 221.
13. Kossel and Neumann, Ber. 1894, 27, 2215.
14. Neumann, Arch. Anat. u. Physiol. 1898, 374; 1899, 552.
15. Altmann, Arch. Anat. u. Physiol., Physiol. Abt. 1889, 524.
16. Feulgen, Zeit. Physiol. Chem. 1914, 90, 261.
17. Levene, Zeit. Physiol. Chem. 1901, 32, 541; 1902-03, 37, 402; 1903, 38, 80.
18. Jones and Austrian, J. Biol. Chem. 1907, 3, 227.
19. Osborne and Harris, Z. Physiol. Chem. 1902, 36, 85.
20. Levene and Mandel, Biochem. Z. 1908, 10, 215.

21. Kossel and Neumann, Ber., 1893, 26, 2753.

22. Kossel and Steudel, Z.Physiol.Chem., 1902-03, 37, 177.

23. Levene, Z.Physiol.Chem., 1903, 39, 479; 1905, 45, 370.

24. Steudel, Z.Physiol.Chem., 1906, 49, 406.

25. Levene and London, J.Biol.Chem., 1929, 81, 711.

26. Levene, Mikeska and Mori, J.Biol.Chem., 1930, 85, 785.

27. Steudel, Z.Physiol.Chem. 1912, 77, 497.

28. Levene and Simms, J.Biol.Chem., 1925, 65, 519.

29. Feulgen, Z.Physiol.Chem. 1919, 104, 189.

30. Levene and Simms, J.Biol.Chem. 1926, 70, 327.

31. Makino, Z.Physiol.Chem., 1935, 232, 229; 236, 201.

32. Bredereck and Köthnig, Ber., 1939, 72, 121.

33. Stenhagen and Teorell, Trans.Farad.Soc., 1939, 35, 743.

34. Gulland and Jordan and Taylor, J.C.S., 1947, 1131.

35. Cohen, J.Biol.Chem., 1942, 146, 471.

36. Schmidt, Pickels and Levene, J.Biol.Chem., 1939, 127, 251.

37. Tennent and Vilbrandt, J.Amer.Chem.Soc., 1943, 65, 424.

38. Hammarsten, Biochem.Z., 1924, 144, 383.

39. Bang, Hofmeister's Beitrage Chem.Physiol.Path. 1903, 4, 331.

40. Gulland, Jordan and Threlfall, J.C.S. 1947, 1129.

41. Mirsky and Pollister, Proc.Natl.Acad.Sci. 1942, 28, 344;
Biol.Symp. 1943, 10, 247.

42. Sevag, Lackmann and Smolens, J.Biol.Chem. 1938, 124, 425.

43. Signer, Caspersson and Hammarsten, Nature 1938, 141, 122.

44. Hofmann and Osgood, J.Biol.Chem., 1938, 124, 347.

Selected Works of Reference and Reviews.

Levene and Bass. "Nucleic Acids" Chemical Catalog. Co. 1931.

Jones "Nucleic Acids" Longman's, Green & Co. 1920.

Gulland J.C.S. 1938, 1722.

Gulland J.C.S. 1944, 208.

Symposia of the Society for Experimental Biology, No.1.
Nucleic Acid, Cambridge 1947.

CHAPTER II.

The Viscosity of Macromolecular Systems.

Section (i) General Theory of Viscous Solutions.

Section (ii) Types of Viscometer in General Use.

Section (iii) The Frampton Viscometer

Section (iv) The Presentation of Viscosity Data.

Section (v) Streaming Birefringence

Section (i) General Theory of Viscous Solutions.

The problem of the relation between the viscosity of a colloidal solution and the nature of the solute particles is of great interest in view of the possibility of obtaining from viscosimetric measurements some idea of the relative dimensions of the individual particle.

The first step towards the hydrodynamical theory of such solutions was the Einstein equation (1, 2), usually stated in the form:

$$\eta/\eta_0 = 1 + KC_v$$ where η = viscosity of solution, η_0 = viscosity of pure solvent, and C_v = volume fraction of solute and is the product of the weight concentration and the specific volume and where K is a factor related to the shape of the particle. For spheres, Einstein calculated K to be 2.5, and the equation holds good under these conditions over a short range of concentration for some hydrophobic colloids and also for a few proteins.

The viscosities of many biocolloids, however, deviate greatly from the values demanded by the Einstein equation, and in some cases, the viscosity varies with the rate of shear, being anomalous, or structural in character. Other physical evidence indicated that the molecules of such biocolloids

were asymmetric in shape, and there was thus the possibility that the anomalous viscosity was due to the influence of the asymmetric solute particles on the flow properties of the solutions.

The problem of the flow of a solution of ellipsoidal particles has been the subject of many investigations by hydrodynamical methods, and a variety of results has been obtained, according to the particular system considered.

Jeffery (3) and Eisenschitz (4), on the assumption that the particles were so large that the disorientation caused by Brownian movement was a relatively slow process, and that a regular precessional movement of the particles was occurring, calculated a maximum and a minimum viscosity, the former occurring with random orientation of the ellipsoids, and the latter when these had been orientated into the position of minimum energy dissipation. This occurred when the rod-like ellipsoids were rotating about their long axes, parallel to the direction of shear, i.e. a 'rolling' motion, parallel to the central axis of a coaxial viscometer, or at right angles to the direction of flow in a capillary instrument. This was the first hydrodynamical explanation of the phenomenon of structural viscosity.

The two equations derived by Jeffery are:

$$\eta_{min} = \eta_o(1 + 2c_v)$$

and $$\eta_{max} = \eta_o\left[1 + \left(\frac{b/a}{2\left(\ln\frac{2b}{a} - 3/2\right)} + 2\right)\right] c_v$$

b/a = ratio of long to short axis of molecule.

This conclusion as to the orientation was proved experimentally by Taylor (5) for moderately asymmetric particles, i.e. those whose axial ratio was about 3:1.

Guth (6) pointed out, however, that for particles of greater axial ratio, such orientation did not occur, but that the particles became oriented in the direction of shear, i.e. parallel to the direction of flow in a capillary. The problem of relating the observed viscosities to particle asymmetry in this case has been studied by Burgers (7), Eisenschitz (8), and Simha (9), but an unambiguous solution has not been found. Thus for this case of large asymmetric particles, relatively undisturbed by Brownian movement, the hydrodynamical theory is not fully adequate, as Eirich (10) has pointed out in a comprehensive review.

For the opposite case of small anisometric particles, subject to the rapid disorientation of Brownian movement, and accordingly not showing the phenomenon of realisable viscosity variations, Burgers (11) obtained the equation:

$$\eta_{sp} = \frac{4\pi}{3}(ab^2c_v) \cdot \left[\frac{(b/a)^2}{15} - \frac{1}{\ln\left(\frac{2b}{a}\right) - 1.80}\right]$$

Where η_{sp} = specific viscosity, defined as

$\eta_{sp} = (\eta_{rel} - 1)$, where η_{rel} is the relative visocsity; while Kuhn (12) derived more simply an approximate form:

$$\eta_{sp} = \left(2.5 + \frac{(b/a)^2}{16}\right) \cdot c_v$$

Other equations have been derived by Huggins (13, 14) and for the case of overwhelming Brownian movement (and hence random orientation) by Simha (15) who formulated the equation:

$$\eta_{sp}/c_v = \frac{(b/a)^2}{15\left(\ln\left(\frac{2b}{a}\right) - \frac{3}{2}\right)} + \frac{(b/a)^2}{5\left(\ln\left(\frac{2b}{a}\right) - \frac{1}{2}\right)} + \frac{14}{15}$$

This relation has received perhaps the widest use for the calculation of axial ratios, being valid for many of the smaller proteins, provided that the velocity gradient is low. It has been applied also to very asymmetric particles, which give structurally viscous solutions, by extrapolating the viscosity values to zero velocity gradient: Cohen and Stanley

(16) used the equation in this manner to calculate the axial ratio of the ribonucleic acid split off from tobacco mosaic virus nucleoprotein, and the equation has even been applied, apparently successfully, to the case of the tobacco mosaic virus itself (17), giving values of the molecular dimensions similar to those obtained by more direct methods.

This provides, as Lauffer emphasises (17) a useful check on the accuracy of the assumptions involved in the Simha equation, and appears also to indicate that the use of the equation need not be restricted to those cases where "overwhelming Brownian movement" prevails, (the latter term can hardly be applied to tobacco mosaic virus solutions) and it therefore appears that random orientation is the only restriction, provided the particles are independent.

Polson (18) proposed, with no preliminary assumptions, an entirely empirical relationship:

$$\eta_{sp} = 4C_v + 0.098 \left(\frac{b}{a}\right)^2.$$

He considered this equation to be valid for those systems where the particles are moderately large and asymmetric, such that streaming birefringence and structural viscosity are not shown under realisable experimental conditions. Many of the

proteins come within this classification, and, as Svedberg (19) has pointed out, sedimentation data would become superfluous if this equation were general; viscosity and diffusion data could supply a complete picture of the size and shape of the molecule.

As was pointed out by Burgers (11) and also by Eirich (10) the hydrodynamic treatment of the increase in viscosity caused by suspended particles leads to the general equation:

$$\eta_{sp} = Kc_v$$

where K depends on the nature of the particle and the amount of Brownian movement to which it is subject.

For spheres: $K = 2.5$

while for long rod-like particles, where orientation is prevented by more or less intense Brownian movement,

$$K = \frac{(b/a)^2}{15(\ln(\frac{2b}{a})-1.5)} + \frac{(b/a)^2}{5(\ln(\frac{2b}{a})-\frac{1}{2})} + \frac{14}{15}$$

and for the final case, of similarly moderately asymmetric particles whose size is such that orientation is not prevented by Brownian movement, the equation evolved by Jeffery (3) applies, and

$$K = \frac{(b/a)}{2(\ln \frac{2b}{a})-1.5} + 2$$

Some consequences of these general relations may now be considered. For a comparison of the viscosities of solutions of long rod-like molecules, the volume, and the length of the particle will be proportional, in the first approximation, to the molecular weight. Accordingly, the two latter equations may be reduced to the forms: $\eta_{sp} = KM^2C_v$, where M = molecular weight of the particle, and $\eta_{sp} = K'M\,C_v$, respectively.

The last equation is identical with the well-known empirical relation devised by Standinger (20) and its derivation is an indication of the great care that must be used in its application.

A further consequence of these generalised viscosity equations is of interest. The general relation between two variables:

$$x = A + By + Cy^2 + Dy^3 \text{ etc}$$

may be applied to the case of the variation of viscosity with concentration in the form

$$\eta_{rel} = K_1 + K_2C_v + K_3C_v^2 \text{ etc.}$$

K_1 may be identified with the viscosity of the solvent, so the equation

$$\eta_{sp} = \eta_{rel} - 1 = K_2C_v + K_3C_v^2 \text{ etc}$$

is obtained.

It has been shown, however, that all hydrodynamic treatment leads to an equation of the form

$\eta_{sp} = K M^x C_v$, where x has some value between 1 and 2, and it must therefore be concluded that before the results of hydrodynamic theory may be applied to a system, this must be such that the magnitude of the coefficient of the squared concentration term is low, so that the specific viscosity at low concentrations is directly proportional to the volume concentration, i.e. that the intrinsic viscosity $[\eta]$, being the initial value of $\left(\frac{d\eta_{sp}}{dc_v}\right)$, is a constant.

With values of C_v of 10^{-2} and less, the intrinsic viscosity of many lyophilic colloidal systems is a constant, and is characteristic of the system. When this behaviour is not observed, and significant deviations are shown from constant intrinsic viscosity at low concentrations, it must be concluded that other causes in addition to particle asymmetry are in operation. This kind of behaviour has been observed by Edsall and Mehl (21) for the case of the protein myosin, and by Frampton (22) for tobacco mosaic virus nucleoprotein; the latter investigator concluded that in such systems the particles are not dynamically independent, and that the viscous phenomena are due to a type of readily-deformable gel-like structure developing in the solution.

The interpretation of diffusion and viscosity data for such systems is therefore at present obscure, as has been pointed out by Eirich (10) and by Frampton (22)

The possible applications of the hydrodynamic treatment of the viscosity of solutions of asymmetric particles may now be summarised.

For systems where the particles are dynamically independent, and the linear relation between concentration and specific viscosity holds at low concentrations, three cases must be distinguished, according to the absolute size and axial ratios of the particles, and the conditions obtaining in the viscometer. For this purpose, it is convenient to compare (after Simha (15)) the magnitudes of the ratio $K/_{\Theta}$ where K is the velocity gradient in the viscometer, and Θ is the rotational diffusion constant of the particle. This quantity is defined by equations analogous to those of Fick for translational diffusion, and is a measure of the average rotary motion of the particles due to the disorientation of Brownian movement (23, 24). Like the velocity gradient, Θ ~~gradient~~ has the units of reciprocal time. The magnitude of Θ is, of course, determined by the absolute size and degree of asymmetry of the particle.

The three cases to be considered are:

(1) Systems where K/θ is infinite. This implies particles so large and asymmetric that the orientation produced by the velocity gradient is not destroyed by Brownian movement, and two possibilities are evident:

(a) Where the asymmetry is not very great i.e. when the axial ratio is comparatively small. This is the purely hydrodynamic case considered by Jeffery (3), and is characterised by occurrence of realisable maximum and minimum viscosity values for Couette or uniform streaming, and measurements under such conditions can lead to the elucidation of the axial ratios of the particles.

(b) Where the asymmetry of the particle is very marked, and the orientation caused by the velocity gradient is parallel to the direction of shear. No satisfactory hydrodynamic treatment exists for this type of system which again is characterised by the occurrence of structural viscosity. In many cases, streaming birefringence may also be observed at low velocity gradients.

(2) Systems where K/θ is finite. This implies systems in which the regular precessional movement of the particles in flow is disturbed by Brownian movement, and the problem has been examined by Burgers, among others. As Simha (15) has

pointed out, the hydrodynamic theory of such solutions is still incomplete.

Systems of this type show a lesser degree of structural viscosity than those considered under (1)b, and as the particles are subject to the disorientation of Brownian movement, streaming birefringence is not shown at the very low velocity gradients sufficient for the previous case.

(3) Systems where $K/\dot{\theta} \rightarrow 0$. This implies systems where the particles, though asymmetric, are relatively small, and are accordingly subject to overwhelming Brownian movement. No orientation, therefore, is realisable, and neither structural viscosity nor streaming birefringence can be observed. Many synthetic polymers, e.g. polystyrenes, nitro-celluloses, come within this class, the behaviour of which is adequately represented by the equations of Simha.

Section (ii)

Types of Viscometer in General Use.

For the study of solutions of high polymers, it is important to be able to find corresponding values of the shear force and the apparent viscosity, in order that any structural character may be observed; capillary instruments of the Ostwald type are therefore unsuitable.

As has been pointed out by Lawrence, Needham and Shih-Chang Shen (25) the Couette, or co-axial viscometer, in which the solution is placed in the annulus between two cylinders, one of which is rotated relative to the other, presents several advantages. For example, the variation of the shear rate across the annulus of liquid is very small, and equilibrium flow conditions can be attained. The variation of apparent viscosity with shear rate can then be obtained under conditions approximating to ideal.

Although these conditions are not satisfied in capillary instruments, the simplicity, and greater convenience, of measurement have led to the devising of several modifications of the simple capillary method such that simultaneous values of the applied pressure and the apparent viscosity can be obtained. The viscometers of Bingham and Jackson (26) and of Frampton (22) are of this type.

Steady equilibrium flow conditions are not attained in these instruments, and the variation of shear rate across the capillary may be considerable; in consequence, a macromolecular solution of the type (1) or (2) above will undergo greater orientation of the solute particles at the walls than at the centre of the capillary, the velocity gradient in which varies from a maximum at the walls to zero in the centre. Calculations of the mean shear rate from the observed rate of flow can, as Lawrence et al (25) have pointed out, have no significance,

as the orientation of the solute particles within the capillary is not constant, but depends, for each particle, upon its distance from the wall. This renders the quantitative interpretation of results impossible; as, however, it has been shown that the hydrodynamic theory of some systems is at present inadequate, there is no theoretical reason why such instruments should not be used for qualitative investigation of these systems. It will be shown later that the viscosimetric behaviour of sodium deoxyribonucleate solutions provides evidence that this system is of the type referred to.

Section (iii)

The Frampton Viscometer

The modified form of Ostwald viscometer devised by Frampton (22) is of the type discussed above, and has been used throughout the present investigation. With this instrument, corresponding values of the applied pressure and apparent viscosity may be readily obtained, so that it is well suited for the qualitative study of solutions exhibiting structural viscosity. (The particular numerical values obtained with such solutions are of little absolute significance since they depend on the dimensions of the apparatus; with solutions of normal viscosity, this does not, of course, apply).

The instrument consists of a horizontal capillary joined by two wide-bore tubes, these being bent in such a manner that they are close together and parallel, as shown in the diagram, p.32 . The liquid under investigation is added to one arm of the viscometer, and is allowed to flow through the capillary under its own pressure. Observations are made of the relative heights in the two vertical arms at successive intervals of time, until the flow has nearly stopped.

The theory of the instrument has been developed by Frampton. The equation of Poiseuille for the laminar flow of a liquid through a capillary under a pressure difference at the ends of ΔP is

$$V = \frac{\pi r^4 \cdot \Delta P \cdot t}{8 \eta l} \qquad (1)$$

where r = radius of the capillary

t = time of flow of the volume V

η = absolute viscosity

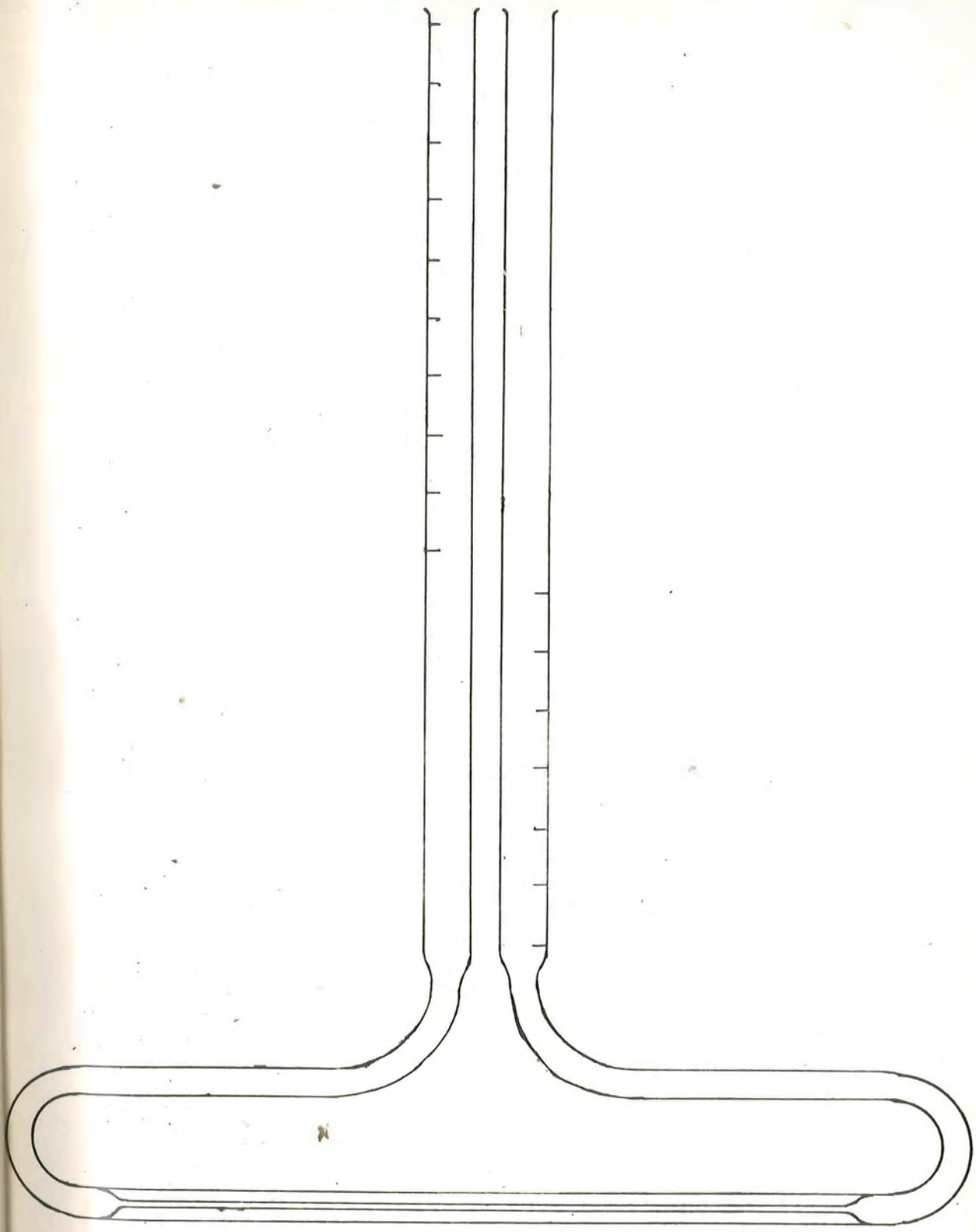
l = length of the capillary

Differentiating to obtain the instantaneous rate of flow:

$$\frac{dV}{dt} = \frac{\pi r^4 \cdot \Delta P}{8 \eta l} \qquad (2)$$

Since in the Frampton instrument, the liquid flows under its own pressure:

$$\Delta P = \rho g h, \qquad (3)$$



THE FRAMPTON VISCOMETER.

where ρ = density of the liquid,

g = acceleration due to gravity,

h = instantaneous difference in height between the two menisci.

$$\therefore \frac{dV}{dt} = \frac{\pi r^4 \rho g h}{8 \eta l} \qquad (4)$$

The total volume of flow, i.e. from the commencement of motion until flow ceases, is given by

$$V = \frac{\pi R^2 h_0}{2} \qquad (5)$$

where h_0 is again the difference in height of the menisci at the commencement of flow, and R is the radius of the vertical arms. Differentiating this equation with respect to h :

$$\frac{dV}{dh} = \frac{\pi R^2}{2} , \qquad (6)$$

and on combining (6) with (4) and rearranging, one obtains the equations

$$\frac{dV}{dt} \cdot \frac{dh}{dV} = \frac{2 \pi r^4 \rho g h}{8 \pi R^2 \eta l}$$

$$\therefore \frac{dh}{h} = \frac{r^4 \rho g}{4 \eta l R^2} dt \qquad (7)$$

Integrating equation (7) between h_1 and h_2, two successive height intervals occurring at times t_1 and t_2 :

$$\ln(h_1/h_2) = \frac{r^4 \rho g}{4 \eta l R^2} (t_2 - t_1)$$

or $$\eta = \frac{r^4 \rho g (t_2 - t_1)}{4 l R^2 \ln(h_1/h_2)}$$

i.e. $$\eta = K \cdot \frac{\rho \cdot \Delta t}{\ln(h_1/h_2)}$$ where K = the constant of the apparatus.

Thus absolute viscosities may be obtained with a knowledge of the dimensions of the apparatus; its main use, however, is the determination of relative viscosities, which are obtained by comparison of the values of $\frac{\rho \cdot \Delta t}{\log(h_1/h_2)}$ for the solution and for the solvent.

Since the pressure is varying continuously during a run, any anomalous character of the viscosity will be revealed as a steady increase in the values of $(\rho \cdot \Delta t / \log(h_1/h_2))$ as the height difference decreases.

Section (iv)

The Presentation of Viscosity Data

Some investigators have presented their results in terms of the intrinsic viscosity, :

$$[\eta] = \eta_{sp}/C_v \quad ;$$

$$(\eta_{sp} = \eta_{rel} - 1)$$

For the classes of solutions where the specific viscosity increment is constant for an appreciable range of concentration, this treatment is ideal, as the intrinsic viscosity is here a constant, characteristic of the particular system. However, for systems which do not obey this condition, the value of determining such a quantity is doubtful; the particular value obtained is not, of course, a constant, and depends on the concentration.

For this reason, and also for simplicity of presentation, the results obtained in the present investigation have in general, been expressed as relative viscosities. Wherever the term "viscosity" is applied, it is the viscosity of the solution relative to that of the solvent that is inferred.

Demonstration of Structural Character.

In a co-axial viscometer, the apparent viscosity may be plotted against the rate of shear, this quantity being determined by the thickness of the annulus of liquid, and the rate of rotation. By plotting the apparent viscosities against the corresponding shear rates, the structural character may be demonstrated.

In a capillary viscometer, however, as has been shown earlier, the rate of shear varies across the capillary. In order to obtain some function of the shear rate by which structural viscosities might be adequately represented when determined in capillary viscometers, Kroepelin (27) devised

a quantity " $\bar{\beta}$ " which he termed the mean velocity gradient, and which was a measure of the shear rate in the capillary:

$$\bar{\beta} = \frac{8V}{3\pi r^3 t}$$

where the symbols have their previous significance.

Kroepelin, and also Philippoff (28) found that, for some solutions showing anomalous viscosity, the measured apparent viscosity was a unique funtion of $\bar{\beta}$.

Edsall and Mehl (21) considered that, though not theoretically free from objection, $\bar{\beta}$ -relative viscosity curves were the best method of representing the structural viscosities of solutions such as that of myosin; Greenstein and Jenrette (29) also used this method of presentation for results obtained with sodium deoxyribonucelate solutions.

In view of the observations of Lawrence et al (25) on the conditions of orientation occurring in a capillary, which have been discussed previously, this practice has not been followed in the present investigation, and all structural viscosities are represented by plots of the apparent relative viscosity against the hydrostatic pressure applied. This last quantity was taken as the geometric mean of the two successive pressure differences between which the viscosity was calculated.

Section (v)

Streaming Birefringence.

The phenomenon of streaming double refraction, i.e. the transformation of plane polarised into elliptically polarised light by passage through a streaming liquid, was first associated with the orientation of anisometric particles through the work of Freundlich and others (30) on sols of vanadium pentoxide.

In 1930, von Muralt and Edsall (31) demonstrated intense streaming birefringence in solutions of the muscle globulin myosin, and in subsequent years many natural and synthetic high polymers have been found to exhibit the same phenomenon. The subject has been extensively reviewed by Edsall (32).

In general, streaming birefringence is associated with those solutions which show structural viscosity since both phenomena are due to the orientation of anisometric solute particles; as has been pointed out by Lawrence and others (25), the two quantities vary in the opposite sense: an increase in the shear rate will tend to destroy structural viscosity, as the particles become more aligned in the direction of flow, until a constant viscosity is attained; the streaming birefringence will, of course, be a maximum when this state has been reached. As is the case with viscous solutions, several cases of birefringent solutions can be distinguished, according to the absolute size and the axial ratios, of the particles, and the

corresponding degree of Brownian movement. The amount of streaming birefringence at specified velocity gradients can be correlated with viscosity data to give information about the average degree of orientation of the particles, as was done by Robinson (33) in 1939. For this purpose a co-axial instrument is required, to give constant and reproducible shear rates; Lawrence, Needham and Shih-Chang Shen (25) have studied the streaming birefringence of protein solutions simultaneously with the measurements of viscosity, in the one coaxial cell, an arrangement which is probably ideal.

Since, in the present investigation, capillary instruments were used for observations of viscosity, for the reasons already outlined, quantitative measurements of double refraction would serve little purpose; accordingly, qualitative observations only were made upon the solutions which were studied viscosimetrically.

For this purpose, a very simple form of apparatus was found to be adequate; a small circular cell on the stage of a polarising microscope, with an arrangement for mechanically stirring the contents, was very satisfactory. The Nicol prisms of the microscope were set for total extinction, and on stirring the solution on the stage, streaming birefringence was readily observed as streaks of light on a dark field.

REFERENCES TO CHAPTER II.

1. Einstein, Ann. Physik, 1906, 19, 289.
2. Einstein, Ann. Physik, 1911, 34, 591.
3. Jeffery, Proc. Roy. Soc. (London), 1923, A102, 163.
4. Eisenschitz, Physik. Z. 1933, 34, 411.
5. Taylor, Proc.Roy.Soc. London A103 58
6. Guth, Kolloid Z. 1936, 74, 147.
7. Burgers, 2nd Report on Viscosity and Plasticity, Amsterdam, 1938, P134.
8. Eisenschitz, Zeit.Physik.Chem. 1933, 103.
9. Simha, Unpublished Work, refrrred to in ref. 15.
10. Eirich, Reports on Progress in Physics, 1940, 7, 329.
11. Burgers, 2nd Report on Viscosity and Plasticity, Amsterdam, 1938, p.183.
12. Kuhn, Kolloid Z. 1936, 74, 147; 75, 15.
13. Huggins, J.Phys.Chem. 1939, 43, 439.
14. Huggins, J.Phys.Chem. 1938, 42, 911.
15. Simha, J.Phys.Chem. 1940, 44, 25.
16. Cohen and Stanley, J.Biol.Chem., 1942, 144, 589.
17. Lauffer, Chem. and Eng. News 1946, 24, 2896.
18. Polson, Nature, 1936, 137, 740; Kolloid Z. 1939, 88, 51.
19. Svedberg, Proc.Roy.Soc. London 1939, B127, 1.
20. Staudinger, Die hochmolekularen Verbindungen. J.Springer, 1932, p.96.

21. Edsall and Mehl, J.Biol.Chem. 1940, 133, 409.

22. Frampton, J.Biol.Chem. 1939, 129, 233.

23. Boeder, Z.Physik. 1932, 75, 258.

24. Edsall, in Cohn and Edsall, Proteins, Amino Acids and Peptides, Reinhold 1943, p.506.

25. Lawrence Needham and Shih Chang Shen. J.Gen.Physiol., 1944, 27, 201.

26. Bingham and Jackson, Bull.U.S. Bureau of Standards 1918-19, 14, 59.

27. Kroepelin, Kolloid Z. 1929, 47, 300.

28. Philippoff, Kolloid Z. 1936, 75, 143.

29. Greenstein and Jenrette, J.Natl.Cancer Inst. 1940, 1, 77.

30. Freundlich, Stapdfeldt and Zocher. Z.Physik Chem. 1924-25, 114, 161, 190.

31. Von Muralt and Edsall, J.Biol.Chem. 1930, 89, 315, 351, Trans.Farad.Soc. 1930, 26, 837.

32. Edsall, in, Advances in Colloid Science, Interscience 1942, Vol.1, p.269.

33. Robinson, Proc.Roy.Soc. London 1939, A170, 519.

CHAPTER III.

The Viscosimetric Properties of the Sodium Deoxyribonucleate of Calf Thymus.

Section (i) Results obtained in Previous Investigations

Section (ii) The Variation of Viscosity with Sodium Deoxyribonucleate Concentration, and the Classification of Viscosimetric Type.

Section (iii) The Effect of Neutral Salts.

Section (iv) The Effect of Acid and Alkali.

Section (v) The Reversibility of the Changes Caused by Acid and Alkali.

Section (vi) The Behaviour of Precipitated Acid-and Alkali-treated materials.

Section (vii) The Behaviour of some other samples of Sodium Deoxyribonucleate.

Section (viii) The Behaviour of certain substances derived from Sodium Deoxyribonucleate.

Section (i)

Results obtained in Previous Investigations

Previous investigators have reported varying results for the viscosity of sodium deoxyribonucleate solutions; it is probable that this is due not only to differing viscosimetric techniques (as stated in Chapter II, it is not possible to obtain exactly corresponding values with different instruments) but also to the varying degrees of degradation of the materials investigated.

Thus Vilbrandt and Tennent (1) report a relative viscosity of 5.9 in the presence of 1% sodium chloride for a 0.3% solution of sodium deoxyribonucleate prepared by the Hammarsten-Bang process, this value being obtained in an Ostwald viscometer; Greenstein and Jenrette (2) using a capillary viscometer of the type designed by Bingham and Jackson (3), in which structurally viscous solutions may be studied, obtained values between 8 and 25, depending on the pressure for a 0.25% solution. The latter value, corresponding to a pressure of 16 cm of water may be compared with the value of 49 obtained with the material used in the present investigation at the same pressure and a concentration of 0.24%. In general, however, the pressure range covered in the present investigation was considerably lower than that used by Greenstein and Jenrette, so that higher viscosities are obtained, and their

structural character manifested to a greater degree, as is evident from, for example, the curves of Graph 3/1, in which the relative viscosities of solutions of varying concentration of nucleate are plotted against the corresponding applied pressure. The solutions showed intense streaming birefringence when studied in the apparatus described in Chapter II.

It is evident that the viscous characteristics of this sample differ widely from those reported by the above investigators; the experiments described in this chapter were performed in order to characterise this sample and relate, if possible, its viscosimetric behaviour and the differencesfrom previous materials to the macromolecular structure of nucleic acid.

Section (ii)

The Variation of Viscosity with Sodium Deoxyribonucleate Concentration, and the Classification of Viscosimetric Type.

In Chapter II it was shown how viscous solutions may be classified according to certain characteristics, among which the degree of structural character, the shape of the viscosity-concentration curve, and the amount of streaming birefringence were the most important.

Experiments were therefore performed in order to effect such a classification. A whole series of solutions was prepared, in which the concentration of the material ranged from very nearly zero to the maximum which could be studied.

The viscosity-pressure relation, i.e. the degree of structural character, was obtained for each, and from this family of curves a viscosity-concentration relation was plotted. The series of viscosity-pressure curves is shown in Graphs 3/1 and 3/2, and the viscosity-concentration relation, at two different pressures, is shown in Graph 3/3. Streaming birefringence was shown at all concentrations.

It is evident that the viscosity increases very rapidly with concentration, solutions of concentration greater than 0.5% being too viscous for study with the range of instruments in use.

In this particular case, a plot of the intrinsic viscosity (taken as η_{sp}/C_v) against the corresponding volume concentration was drawn. The specific volume of the sodium deoxyribonucleate was taken as 0.60 cc/gm., this value being the average of several pycnometric determinations. The resulting curve is shown in Graph 3/4. It is evident that the curve is not linear, and is convex to the viscosity axis.

It was shown in Chapter II that systems whose viscous phenomena are due to particle asymmetry must have a linear relation between the specific viscosity and the volume concentration, i.e.

$$\eta_{sp} = K M^{x} . C_v$$

Consequently, such solutions have a constant and characteristic

intrinsic viscosity $[\eta]$ over an appreciable (low) range of concentration:

$$[\eta] = \eta_{sp}/C_v = K M^x$$

and a plot of the intrinsic viscosity against the volume concentration would yield a straight line, parallel to the concentration axis.

The behaviour of this sample of sodium deoxyribonucleate is in clear contrast with this. The intrinsic viscosity is not independent of the concentration even when this has very low values, and the curve slopes in a non-linear manner. Moreover, the equation of the initial slope of the curve, up to a volume concentration of 0.0012 is

$[\eta] = 12{,}000 + 2.5 \times 10^7 C_v$ approximately. (This value (12,000) of the intrinsic viscosity at zero concentration may be compared with the value of about 3,500 obtained by Greenstein and Jenrette (2) for one of their materials). The relation of the specific viscosity to the volume concentration is therefore:

$\eta_{sp} = 12{,}000C_v + 1.25 \times 10^7 C_v^2$, a relation which can of course, be obtained from the relative viscosity concentration curve.

The very great magnitude of the coefficient of C_v^2, with

consequently great variation in the value of the intrinsic viscosity is conclusive proof that the high viscosity of sodium deoxyribonucleate solutions is due to causes other than the undoubtedly high molecular weight and degree of particle asymmetry; it must therefore be inferred that at all realisable concentrations, a very high degree of particle interaction is occurring.

Section (iii)

The Effect of Neutral Salts.

Greenstein and Jenrette (2), working with two samples of sodium deoxyribonucleate prepared by the Hammarsten-Bang technique, investigated the effect of many uni-univalent electrolytes upon the viscosities. In all cases, there was an instantaneous drop in the viscosity, which was proportional to the salt concentration.

With one sample of sodium deoxyribonucleate, at a concentration of 0.50%, the addition of sodium chloride to a concentration of 4M led to a very great reduction in the structural character and magnitude of the viscosity, and the amount of streaming birefringence was similarly reduced. In the presence of 6M sodium iodide or 8M guanidine hydrochloride the structural character of the viscosity was completely destroyed, and the streaming birefringence was diminished to a value below the limit

of detection with their apparatus. With another sample of sodium deoxyribonucleate which gave an initially more viscous solution, and which was more strongly birefringent, some degree of structural character and birefringence was detectable at all salt concentrations. A striking feature of the salt effect was its ready reversibility, i.e. if the salt were removed by dialysis, or by precipitation of the sodium deoxyribonucleate followed by re-solution, the viscosity of the salt-free solution approximated closely to that of the original.

It has already been shown that the viscosity values of solutions to the sodium deoxyribonucleate used in the present investigation are considerably higher than any previously recorded; accordingly it was anticipated that the effect of added salts might be different from that reported for other materials.

The effect of (a) Sodium Chloride, and (b) guanidine hydrochloride upon the viscosity of this sample was investigated.

These salts were chosen (a) because it was proposed to study the effect upon the viscosity of varying the pH, by using varying quantities of sodium chloride and hydrochloric acid, and of sodium chloride and sodium hydroxide,

when a knowledge of the effect of sodium chloride would be essential, and (b) because Greenstein and Jenrette reported the guanidinium ion to be the most effective in reducing the viscosity and streaming birefringence of sodium deoxyribonucleate solutions. The efficacy of this ion in denaturing proteins is well known and the two effects have a superficial similarity.

The results of this investigation are shown in the graphs 3/5, and 3/6, which demonstrate the structural viscosities of a 0.24% solution of sodium deoxyribonucleate in the presence of varying quantities of sodium chloride and of guanidine hydrochloride, respectively. Graph 3/7 shows the variation of the relative viscosities at a constant pressure of 8,000 dynes/sq.cm. with sodium chloride and guanidine hydrochloride concentrations.

The initial drop in viscosity when the salts were added took place too rapidly to be studied; the subsequent value remained sensibly constant over a considerable period.

It is evident from the graphs that the effects of the two salts are very similar; in both cases, very low concentrations of salt have a considerable effect upon the viscosity, this, in the presence of M/1000 salt being noticeably less than that of the original salt-free solution. There is a fairly steady drop in viscosity with increased

salt concentration until the latter is about M/100, when, until the concentration reaches M/2, further increments have little effect.

Between 0.5 and 1.5M, an irregularity occurs, the viscosity reaching a sharp maximum at about 1.0M in each case. When the salt concentration is increased beyond this figure, the viscosity drops again to its value between 0.01 and 0.5 M, and is maintained at this value until the salt is saturated, at about 5M in the case of sodium chloride and 8M for guanidine hydrochloride.

The structural character of the viscosities became less as the salt concentration was increased, as is evident from the slope of the curves; at values greater than 0.01 M, however, the solutions had approximately the same degree of structural character. The increase in magnitude of the viscosity at 1M salt concentration is reflected in an increase of structural character (lesser slope of the viscosity-pressure curves) at this point.

At all concentrations studied, the solutions were strongly birefringent, an approximate indication of the relative amounts being shown in Table I.

Table I.

Salt Concentration.	Relative Amount of Streaming Birefringence(0.24% sodium deoxyribonucleate)
0	4
0.002 - 0.01	3
4M(NaCl),6M(Guanidine HCl)	2 or 3

It can be seen that the constant minimum viscosity, attained at salt concentrations 0.01 to 0.50M, and greater than 2M, is the same in both cases, about 30 at a pressure of 8,000 dynes/sq.cm; also, the maximum viscosity, shown at about 1M, was about 40-50 in each case. It is strongly inferred, therefore, that the salts are exerting identical actions upon the macromolecular structure in solution, a conclusion which is not in agreement with that of Greenstein and Jenrette, whose results showed different viscosities for corresponding concentrations of the two salts, and a constant decrease in viscosity with increased salt concentration.

These investigators came to the tentative conclusion that the above phenomena indicated a depolymerisation of the very asymmetric macromolecule to smaller, less asymmetric particles, a process which could be reversed by removing

the salt. They later (4) modified this conclusion in the light of osmotic pressure determinations. In any case, whatever action does occur cannot involve a rupture of the internucleotide phospho-ester linkages, as the electrometric titration curves of the material in water, in 4M sodium chloride and in 2.53M guanidine sulphate are identical, showing that no new groups have been liberated. These, and all subsequent electrometric titrations referred to, were carried out by Dr. H.F.W. Taylor, to whom the author is greatly indebted.

Section (iv)

The Effect of Acid and Alkali

It was early realised that acid or alkaline treatment reduced the viscosity of sodium deoxyribonucleate solutions, but the inference that such treatment should be avoided in the extraction process, was not appreciated until comparatively recently.

Thus Jones and Austrian (5) reported that the viscosities of solutions of sodium deoxyribonucleate prepared by the Neumann technique decreased when acid or alkali were added. Their observations were essentially qualitative, the viscosities being compared by timing the rates of flow through a particular pipette.

Hammarsten, in his original paper (6) on the physical properties of sodium deoxyribonucleate prepared by mild methods, reported that the viscosity of this material dropped considerably when hydrochloric acid was added, and found that when the solution had been treated with alkali and then neutralised, gelling occurred. He considered that this treatment resulted in the production of the " α salt of thymus nucleic acid" reported by Neumann (7), who distinguished between two types of nucleic acid " α " and " β " by this ability to form a gel. The gel-forming, or "α" type, was considered to be less degraded than the "β" form, whose solution did not gel with this treatment.

The first quantitative observations on this phenomenon were published by Vilbrandt and Tennent (1), who worked with a material prepared by Caspersson using the Hammarsten-Bang technique. They measured the viscosities of 0.3% solutions of varying pH in the presence of 1% sodium chloride. All viscosity determinations were performed with an Ostwald viscometer; accordingly variations in the structural character could not be examined.

Under these conditions, they found that the solution of sodium deoxyribonucleate had a maximum viscosity of 5.9 at pH 5.6, and that the viscosity fell fairly sharply, with

no well-marked point of inflection, to values approximating to that of the solvent when the pH's 2.6 and 11 were attained.

On neutralising the solutions which had been alkali-, or acid-treated, they found a slow increase of viscosity, reaching a maximum value of about half that of the original solution after an interval of 5 - 10 hours. Parallel ultracentrifugal experiments (8) indicated a slow increase of the sedimentation constant.

This behaviour was ascribed to a rapid splitting of the originally long polynucleotide chain into a series of much smaller fragments, which, on neutralisation, slowly re-polymerised to form a system of widely varying molecular sizes. These investigators reiterated the importance of mild methods of extraction for nuclear materials, in order that experimental results be valid for an understanding of cell processes.

The results of Vilbrandt and Tennent's work thus showed that neutralisation after acid or alkaline treatment did not yield a product identical with the original material; accordingly, it seemed probable that the exceptionally high viscosity of the sample of sodium deoxyribonucleate used

in the present investigation was due to the care taken to maintain a very nearly neutral reaction during its extraction. The effect of alkaline and acid treatment on the viscosity of this sample was therefore investigated, using the same technique as before, so that structural viscosities were obtained. Comparative measurements of the degree of streaming birefringence at the various pH's were also performed. A constant ionic strength of 0.01 (due only to the ions Na^+, Cl^-, H^+ or OH^-) was preserved throughout, since the effect of sodium chloride alone on the viscosity reaches a constant value at this figure.

The structural viscosity curves at the various pHs are shown in Graph 3/8. Curve I on this graph is a composite curve drawn through the points obtained at nine different pHs, giving each point approximately equal weight. It will be observed that it is very close to the best curve that could be drawn through the points obtained with the original sodium deoxyribonucleate solution in the presence of 0.01 M salt alone. Graph 3/9, in which the viscosities at two different pressures are plotted against the particular pH at which they were obtained, demonstrates the striking effect of acid and alkali more clearly. It is evident from the remarkable shape of this curve that the viscosimetric behaviour of this sample of sodium deoxyribonucleate is different from that reported by Vilbrandt and Tennent;

the high value of the viscosity at neutrality is maintained until the critical pHs 5.3 on the acid, and 10.8 on the alkaline side, are reached, when the viscosity drops very rapidly to values near that of the solvent.

The structural character of the viscosity, as shown by the slope of the viscosity-pressure curves in Graph 3/8, undergoes a corresponding change: at pHs between 5.3 and 3.5, and between 10.8 and 12.1, there is a progressive decrease in the degree of pressure-variation, and at the lowest and highest pHs the amount of structural character is negligible.

Similar behaviour is also evinced by the streaming birefringence, the relative values of which at the different pHs are shown in Table II.

Table II.

Variation with pH of the degree of streaming birefringence of sodium deoxyribonucleate ("NaDN") / solution.

pH	3.7	4.0-4.3	4.3-5	5-10	10-10.7	10.9-11.6	12.0
Relative amounts of streaming birefringence on arbitrary scale.	0	1	2	3	2	1	0

The NaDN concentration was 0.24% and the ionic strength 0.01 throughout. The relative amount of streaming birefringence shown by the material in water at pH 7 was 4.

The approximately constant amount of streaming birefringence shown by solutions of pH 5 to 10 undergoes a rapid decrease at the critical pHs, and is too slight to be observed in the conditions of the experiment when the pHs 3.7 and 12.0 have been attained.

Thus at the critical pHs of 5.3 and 10.8, there is a fundamental change in the macromolecular structure of sodium deoxyribonucleate, demonstrated by the loss of high structural viscosity and streaming birefringence. This change at certain critical pHs is evidently closely related to the acid-base characteristics of the material, and further information as to its nature may be obtained from the electrometric titration data of Gulland, Jordan and Taylor (9).

The base-binding curve for this sample of sodium deoxyribonucleate is shown in Graph 3/10. It can be seen that there is a marked discrepancy between the initial curves upwards and downwards from pH7, and the corresponding back-titration curves from pH 2.8 and pH 12. These back-titration curves are "normal", i.e. they follow closely the theoretical curve demanded by the Henderson-Hasselbach equation for the various groups known to be present. The initial curves from pH7, however, are not coincident with the back-curves; the sodium deoxyribonucleate is unbuffered between pH 5.6 and 10.9, but at these pHs, which are very

close to the critical values at which the viscosity commences to drop, there are sharp points of inflection, and a liberation of titratable groups occurs. This liberation is complete at the pH values 3.3 and 12.2, when initial and back-titration curves are coincident.

This behaviour has not previously been reported for any other nucleic acid, the initial and back-titration curves of, for example, commercial yeast ribonucleic acid being coincident over the whole range (if allowance is made for the slight amount of hydrolysis that occurs at the alkaline end.)

It was shown by Gulland, Jordan and Taylor that the groups titrating over the pH range 2.5 - 6, and 8.5 - 12 were the amino groups, and the enolic hydroxyl groups, respectively, of the purine and pyrimidine radicals; as stated, the back titration curves for this sample of NaDN are in close agreement with the theoretical curves for these groups.

It must therefore be concluded that the amino- and the enolic hydroxyl groups of the purine and pyrimidine radicals are blocked or "masked" in some manner in the original sodium deoxyribonucleate and that treatment with acid or alkali destroys the blocking and liberates the groups, which are then titratable in the normal manner. Furthermore, it

must also be inferred that the amino and enolic hydroxyl groups are intimately related, as the "liberation" of the amino groups by titration with acid to pH3.5 also liberates the enolic hydroxyl groups which are not titratable by acid; i.e. the titration curve from pH 3.5 upwards follows the back titration curve over the whole range. Similarly, alkaline treatment to pH 11.5 liberates the amino groups as well as the enolic hydroxyls, the acid titration curve from this pH then following the back titration curve over the whole range.

The conclusion from this evidence is that the macromolecular state of the sodium deoxyribonucleate undergoes a fundamental and apparently irreversible change at certain critical pHs, the change being such as to remove the characteristics of high viscosity and intense streaming birefringence previously exhibited. The data from electrometric titration indicate that simultaneous changes are occurring in the amino and enolic hydroxyl groups of the molecule, and it therefore seems probable that the complex macromolecular structure of the material is in some way dependent upon these particular groups.

In order to elucidate the nature of these changes, further experiments were undertaken: in these, the reversibility of the change was investigated, and the effects of precipitation and re-solution upon the viscosimetric

behaviour were examined.

Section (v)

The Reversibility of the Changes Caused by Acid and Alkali.

Althoughtitration evidence indicated that these changes are irreversible, i.e. there is a discrepancy between the initial and back-titration curves, some degree of reversibility was indicated from viscosimetric evidence: the gelling of solutions on neutralisation after treatment with alkali, first reported by Neumann (7) and confirmed by Hammarsten (6) was regarded as some indication of the state of degradation. In support of this was the fact that materials subjected to more drastic treatment, by boiling with alkali, which were presumably liable to be degraded to a greater extent, failed to get on neutralisation; also there was the evidence, cited earlier, of Vilbrandt and Tennent. The changes occurring in the viscosimetric properties of solutions of this sample of sodium deoxyribonucleate when neutralised after treatment with acid or alkali were therefore investigated.

The same conditions of sodium deoxyribonucleate and sodium chloride concentration (0.24% and 0.01 M respectively) were preserved in the solutions, and structural viscosity determinations were made upon them at various intervals of time after the neutralisation.

(a) Behaviour of a sample neutralised after alkaline treatment to pH 12.5.

The structural viscosity curves obtained after various intervals of time in this case are shown in the Graph 3/11. To demonstrate the effect of lapse of time more clearly, the relative viscosities at pressures of 4 and 8,000 dynes/sq.cm. were plotted against the time interval after neutralisation to give the curve of Graph 3/12.

It will be seen that the viscosity increases steadily with lapse of time and regains its structural character; after four days the viscosity values are of the same order as those of the original, but the system resembles more closely a true gel, in that the viscosity at low pressures is very great. Some degree of thixotropic effect was noticeable, especially in more concentrated solutions subjected to similar treatment. On shaking, the gel was destroyed, and reproducible structural viscosities obtained.

Thus the observations of Vilbrandt and Tennent have in principle been confirmed; the rise in viscosity after four days was approximately the same fraction of the original viscosity before alkaline treatment, but in this case, of course, the viscosity values were approximately ten times as great.

(b) Behaviour of samples neutralised after acid treatment to pH 3.2.

A solution prepared in an analogous manner to the above, having the same concentrations, failed to show any significant increase in viscosity with time, the values after four days being approximately twice that of the solvent. A solution of greater sodium deoxyribonucleate concentration (1.0%) however, was found to gel completely on standing for sixteen hours, the system being thixotropic. The viscosity of the homogeneous solution formed on shaking was too great for measurement with the present range of instruments (with which viscosities between 1 and 1,000 could be studied). On dilution of this solution with an equal volume of water, the structural viscosity curve Va in Graph 3/13 was obtained, and no significant increase from these values took place on standing for five days.

Parallel electrometric titrations were carried out over the same time intervals; in both cases no deviation from the original back-titration curve could be detected. Both acid- and alkali-treated solutions were tested periodically for the re-appearance of streaming birefringence but in no case could this be detected.

The changes which lead to the recovery of high and structural viscosity in solutions that have been alkali-treated and neutralised cannot therefore lead to the form-

ation of a system resembling the original macromolecular structure.

Section (vi)

The Behaviour of Precipitated Acid- and Alkali-treated Materials.

In order to obtain further information as to the nature of the macromolecular changes brought about by acid and alkali, samples of materials were prepared which had been precipitated after subjection to various acid or alkaline treatments followed by neutralisation. Precipitation was effected by pouring the solutions into excess alcohol, when simultaneous precipitation, dehydration and removal of electrolyte occurs.

Four such materials were prepared: a sample of sodium deoxyribonucleate which had been alkali-treated and then neutralised was divided into two halves, one of which was precipitated immediately, and the other after standing for four days at neutrality. Similarly, two acid-treated materials were obtained; the details of all preparations are given in Chapter VIII. The period of four days was chosen in view of the fact that the viscosity of neutralised alkali-treated solutions reaches a maximum after this interval.

The elementary analysis figures of the four materials prepared in this way are shown in Table III, together with

those of the original material "T5/1", and three other samples to be discussed later.

Table III

Material	Treatment	When precipitated	C	H	N	P	Na
T5/1	-	-	35.4	3.6	15.3	9.33	6.9
T5/N3	Alkali	Immed. after neutralisation	35.7	3.8	15.6	9.6	6.4
T5/N1	Alkali	After 96 hours	36.1	3.8	15.4	9.58	-
T5/H1	Acid	Immed. after neutralisation	36.5	4.2	15.5	-	6.1
T5/H2	Acid	After 96 hrs.	35.9	3.8	15.8	9.41	6.3
T/Cas	-	-	35.5	4.1	15.3	-	-
T5/2	-	-	36.0	3.9	15.7	9.39	6.5
TC/1	-	-	-	-	14.5	8.57	6.17

It is evident that the only appreciable analytical differences between these materials and the original lies in their sodium analyses which are all slightly lower. Part of this discrepancy, however, can be attributed to the presence of a small amount of phosphoryl dissociation which has not been neutralised, as is shown by the titration evidence of Gulland, Jordan and Taylor (39). In view of the difficulties encountered in the accurate determination of sodium in organic substances containing phosphorus (see

Chapter VIII), it is doubtful whether the difference in the analyses has any real significance.

Viscosimetric Behaviour.

(a) Alkali-treated materials.

The structural viscosity curves for the materials T5/N3 and T5/N1 at a concentration of 0.24%, alone, and in the presence of 0.01 M sodium chloride are shown in Graph 3/13. It is evident that in all cases the viscosities are much lower than those of the original T5/1 under comparable conditions, and the effect of sodium chloride is once more to reduce the viscosity greatly.

The viscosity of the material which had been allowed to stand before precipitation was considerably greater than that of the other; on standing, however, in the presence of 0.01 M salt, the viscosities of the latter sample rose to values approximating to those obtained for the material which had been precipitated after standing. It appears, therefore, that precipitation followed by re-solution does not greatly interfere with those processes leading to the recovery of high viscosity.

Since the viscosity of the sample which had stood before precipitation was of reasonable magnitude, the effect upon the viscosity of varying the pH was investigated, in the same manner as for the original material. The

structural viscosity curves obtained are shown in the graph 3/14, and the viscosity as a function of pH is shown in graph 3/16, curve 1. The contrast between the shape of this curve, and that for the original is very marked; the sharp points of inflection of the latter are replaced by a gradual curve, the viscosities dropping to low values at pH's less removed from neutrality. The viscosimetric behaviour of this substance, both in magnitude and pH-dependence, shows very great similarities to that reported by Vilbrandt and Tennent (1) for their material "STN-2", and described earlier in this chapter. This substance STN-2, had been prepared by Caspersson using the Hammarsten-Bang extraction process.
There are thus strong indications that these two samples of sodium deoxyribonucleate, T5/N3,and STN-2, are of similar macromolecular structure: the further inferences will be discussed later.

(b) Acid-treated materials.

As was,the case with the alkali-treated materials, the viscosities of the acid-treated samples were much less than that of the original and their structural character was similarly less marked. The structural viscosity curves for the two materials alone, and in the presence of 0.01 M sodium chloride, are shown in the Graph 3/13.

Their behaviour closely resembles that of the alkali-treated samples, the viscosity values for the material which had been allowed to stand before precipitation being greater than the corresponding values for the material precipitated immediately. The viscosity of the latter material, however, did not increase with lapse of time.

The effect of variation of the pH upon the viscosity of the material which had been allowed to stand is shown in Graph 3/16, curve II; this curve resembles the corresponding one obtained for the alkali-treated material, the viscosity values, however, being somewhat lower.

The titration curves of these four materials were sensibly identical. In all cases, some "blocking" of the enolic hydroxyl groups was present, but this was not so marked as in the original, i.e. the back-titration curves of the materials from pH 12, which were identical with the back-curve for the sample T 5/1, did not coincide with the initial curves from pH 7, but the discrepancy was much less than in the original material. No blocking or masking of the amino groups was shown in any case, the initial titration curve to pH3 coinciding with the back curve to pH 7.

All solutions were tested for the presence of streaming birefringence, but in no case could this be detected.

Section (vii)

The Behaviour of some other Samples of Sodium Deoxyribonucleate.

Some other samples of this substance, of varying origin, became available, and were investigated.

(a) "T/Cas." This was a sample which had been prepared by Prof. Caspersson, using the Hammarsten-Bang extraction process, and obtained through the courtesy of Prof. Astbury. In appearance, it was indistinguishable from the material T5/1 used in the present investigation, being white, very fibrous, and forming a clear viscous solution, but positive Sakaguchi and biuret tests indicated the presence of protein. This was removed in the same manner as before, and the analytical values for the protein-free material are shown in Table III on p.63. The analytical similarity to the material T5/1 is at once evident.

The viscosimetric behaviour, however, closely resembled that of the sample "STN-2" supplied to Vilbrandt and Tennent by the same investigator, behaviour which has already been described. That is, the material was very similar to those produced by acid or alkaline treatment of the sample T5/1. The structural viscosity curves obtained for the material alone, and in the presence of 0.01 M sodium chloride are shown in Graph 3/13. The resemblance to the acid and

alkaline treated materials was also demonstrated by its titration curve, which was almost identical with the curve obtained for all the former materials.

(b) "T5/2". This material had been prepared in the same way as the sample T5/1, the substance fully investigated; but owing to impurities in the chloroform used for deproteinisation, the solution had become slightly acid in this stage of the extraction. (The pH recorded was about 5). The solution was neutralised before the final precipitation with alcohol, and the material finally isolated was apparently similar to the previous sample, being white and fibrous, and having similar analytical values. (See Table III, p.63.)

In its viscosimetric behaviour, however, it was different from the sample T5/1; the structural viscosity curves obtained under various conditions are shown in the Graph 3/13, and it is evident that all viscosity values are much lower than those of the previous material under corresponding conditions. The titration curve of the material was also different from that of T5/1, showing much less discrepancy between the calculated, or back-titration curve and the initial curve upwards from pH 7; only very slight blocking of the amino groups was present, the titration curves on the acid side being nearly identical.

(c) "TC/1". This material was a commercial preparation of sodium deoxyribonucleate, prepared by a method involving the use of hot alkali, marketed by Messrs. British Drug Houses Ltd.

In appearance, it was a non-fibrous brownish powder and on stirring with water it gave a clear, nearly colourless solution which was not appreciably more viscous than the solvent.

Solutions were therefore studied by means of an Ostwald viscometer, and the results obtained are shown in the Graph 3/16 which demonstrates the effect of varying the pH upon the viscosity of a 4% solution of the material.

The effect is obviously of a different order of magnitude from those obtained with all previous materials; the analysis (table III, p.63) and the electrometric titration curve, which showed the presence of a considerable amount of secondary phosphoryl dissociation confirm these indications that the material was a greatly degraded sample.

Thus it may be concluded from the viscosimetric behaviour of all these materials, that acid treatment followed by precipitation produces an effect on the macromolecule closely similar to that produced by alkali, and that in both cases, the materials so obtained, possessing analytical identity with the original, and having the same number and type of dissociable groups, are yet very

different from the macromolecular viewpoint. Furthermore, there are strong indications that the Hammarsten-Bang process of extraction leads to the isolation of a more or less degraded material, and that even mildly acid or alkaline extractions result in a material which may differ considerably from that extracted at a neutral reaction.

This conclusion emphasises the necessity, only recently realised in practise, of very mild methods of extraction of materials which are to be investigated by physico-chemical methods, and the great care which must be exercised before it is possible to ascribe physiological roles to complex substances of biological origin.

Section (viii)

The Behaviour of Certain Substances derived from Sodium Deoxyribonucleate.

(a) At this stage in the investigation there became available a substance formed by the reaction of sodium deoxyribonucleate (sample T5/1) with 'mustard gas', / -dichlorodiethyl sulphide. This substance was prepared by Mr. D.T. Elmore, B.Sc. (10). Its analysis showed the presence of 2.2 "H-residues" (i.e. the group $-CH_2-CH_2-S-CH_2-CH_2-$) per four phosphorus atoms, and as it had been precipitated by acid alcohol, was isolated as the free acid.

The electrometric titration curve of the material indicated that three to four amino groups, and one enolic hydroxyl group per statistical tetranucleotide (i.e. per four phosphorus atoms) were blocked; the inference, accordingly, is that, since two H residues block approximately four groups, each H residue is attached at two points to the polynucleotide chain. This can be accomplished either by a residue linking two groups in one chain, or by cross-linking between two neighbouring chains.

The viscosimetric behaviour of this material became, therefore, of great interest, in view of the dependence of the high viscosity of the original material upon the native state of the amino and enolic hydroxyl groups. The possible correlation between the presence of the bridge between these groups in the nucleic acid, and the well-known delayed healing of mustard gas burns also raises a question of great biological interest.

The structural viscosity curves obtained with this substance at two concentrations are shown in the graph 3/17, together with one curve for the original parent material. It is at once evident that the viscosities of the 'H-substance' are greater than those of the original sodium deoxyribonucleate at corresponding concentrations, and, moreover, that the dependence upon the applied pressure is even greater, i.e. the viscosity shows a greater degree

of structural character. The pH of the solution of 'H-substance' was approximately 2, at which value the viscosity of the original is very close to that of the solvent.

(b) Several attempts were made to prepare a deaminated sample of sodium deoxyribonucleate, so that its viscosimetric properties could be investigated. Fletcher, Gulland, Jordan and Dibben (11) had proved in the case of yeast ribonucleic acid that deamination did not necessarily decrease the molecular size, and on the existing evidence for the poly-phospho-ester structure of thymus deoxyribonucleic acid, it seemed probable that this would also prove the case for the latter material.

Deamination was carried out by nitrous acid at various pHs between 3.8 and 4.5 in the presence of acetate buffers for periods up to four days, the solutions being neutralised before precipitation with alcohol. The materials so prepared, which were very slightly yellow in colour, gave extremely viscous solutions; analysis and titration data, however, showed the presence of unaltered amino groups, and it was found that under these conditions, deamination would proceed no further than 50-60% of the theoretical amount. The same result was obtained when alkali-treated samples were used as starting materials.

Deamination under more acid conditions was found to give darker coloured products, yielding non-viscous solutions, which were very deficient in nitrogen. It was therefore concluded that the hydrolysis of the purine-deoxyribose glycosidic link to give thymic acid (12, 9) begins to occur at a pH very close to that at which deamination proceeds to completion. The attempt to prepare fully deaminated samples was therefore abandoned.

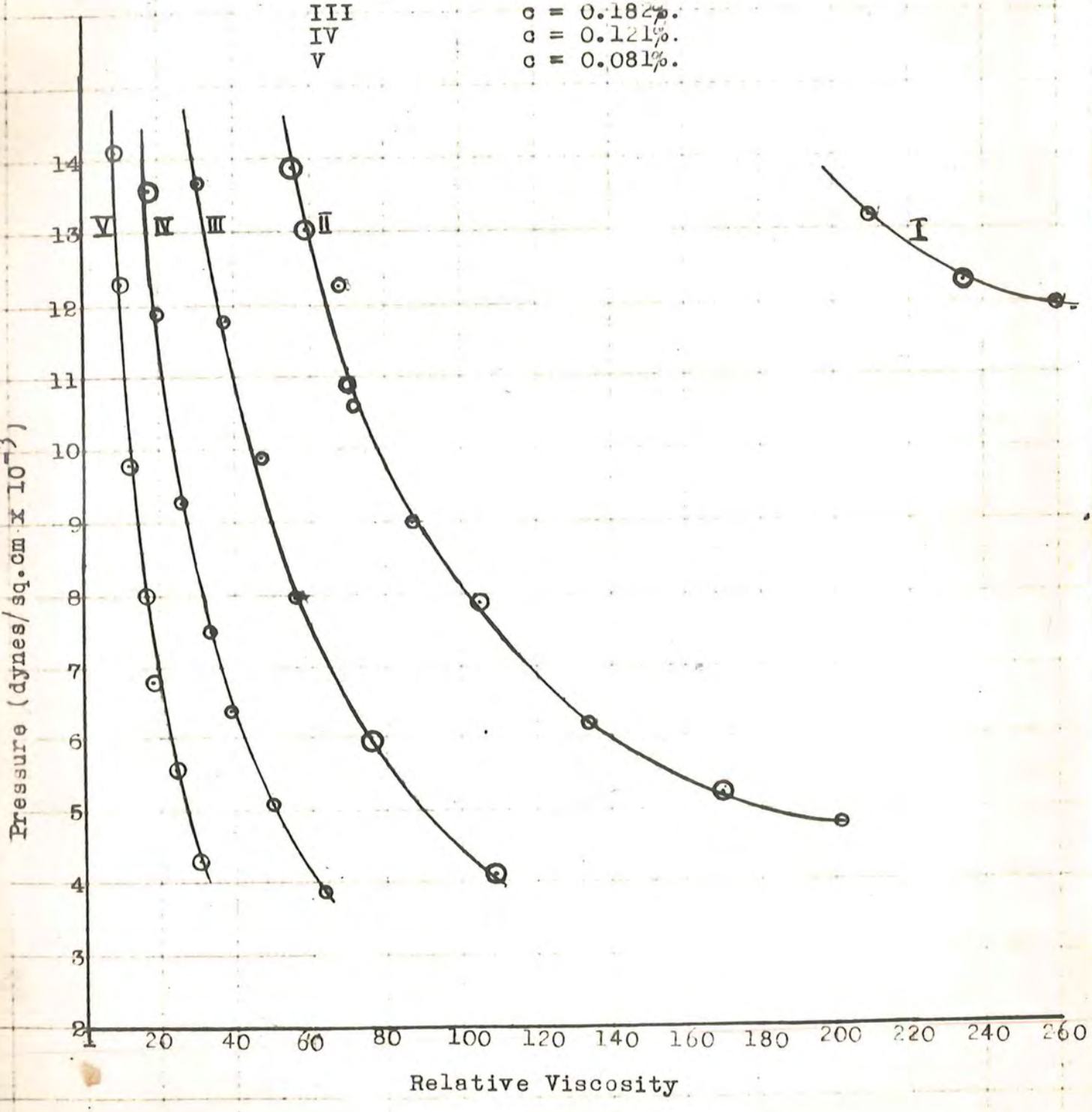
REFERENCES TO CHAPTER III

1. Vilbrandt and Tennent J.Amer.Chem.Soc. 1943, 65, 1804.
2. Greenstein and Jenrette J.Natl.Cancer Inst. 1940,1,77.
3. Bingham and Jackson Bull.U.S.Bureau of Standards 1918-19, 14, 59.
4. Greenstein and Jenrette J.Biol.Chem. 1943, 150, 107.
5. Jones and Austrian, J.Biol.Chem. 1907, 3, 1.
6. Hammarsten Biochem.Z. 1924, 144, 383.
7. Kossel and Neumann, Ber. 1894, 27, 2215.
8. Tennent and Vilbrandt, J.Amer.Chem.Soc. 1943, 65, 424.
9. Gulland, Jordan and Taylor, J.C.S., 1947, 1131.
10. Elmore, Gulland, Jordan and Taylor, Biochem.J. in press.
11. Fletcher, Gulland, Jordan and Dibben, J.C.S. 1944, 30.
12. Feulgen, Z.Physiol.Chem. 1918, 101, 296.

GRAPH 3/1

Structural Viscosity Curves at Different Concentrations I

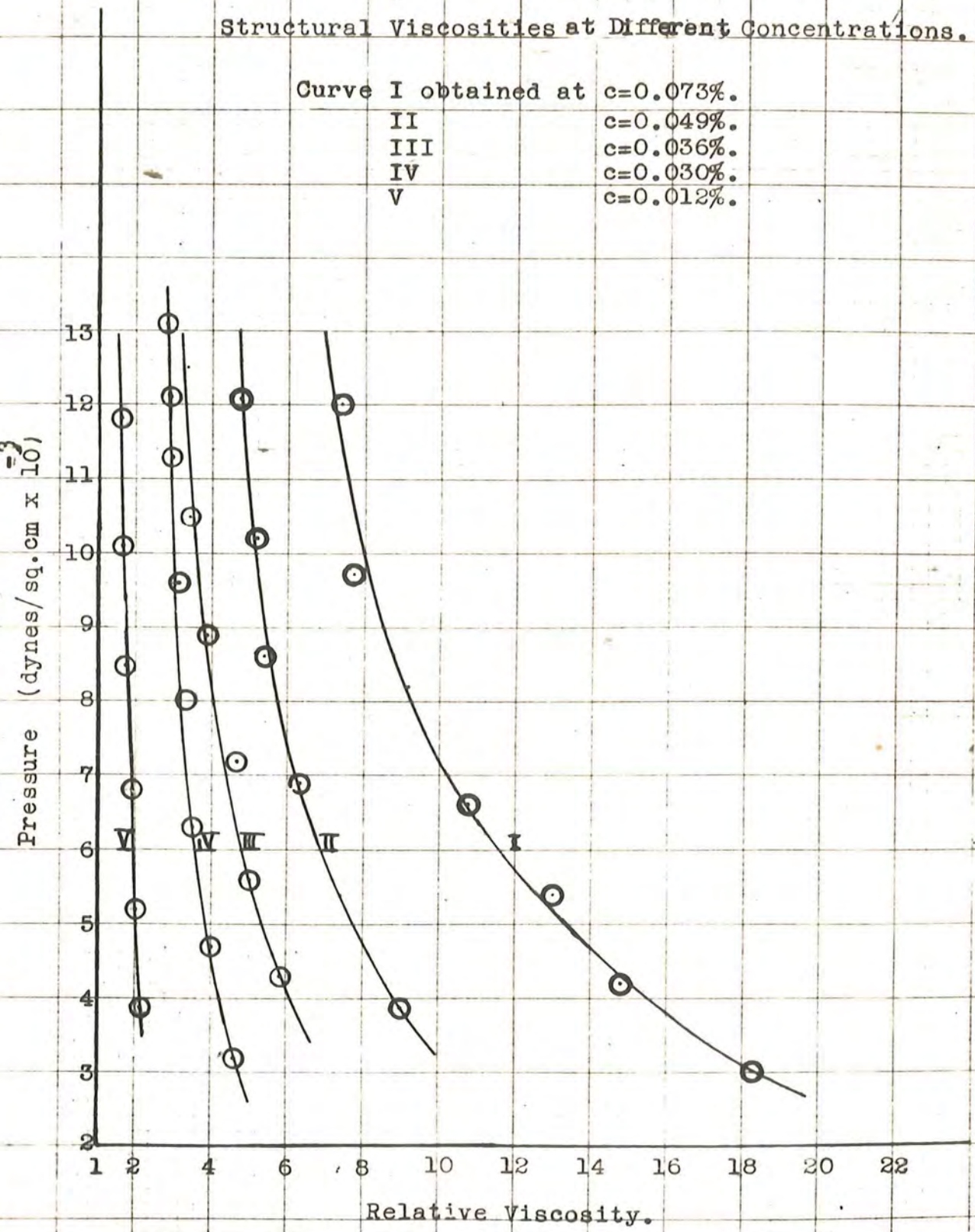
Curve I obtained at c = 0.364%.
II c = 0.243%.
III c = 0.182%.
IV c = 0.121%.
V c = 0.081%.

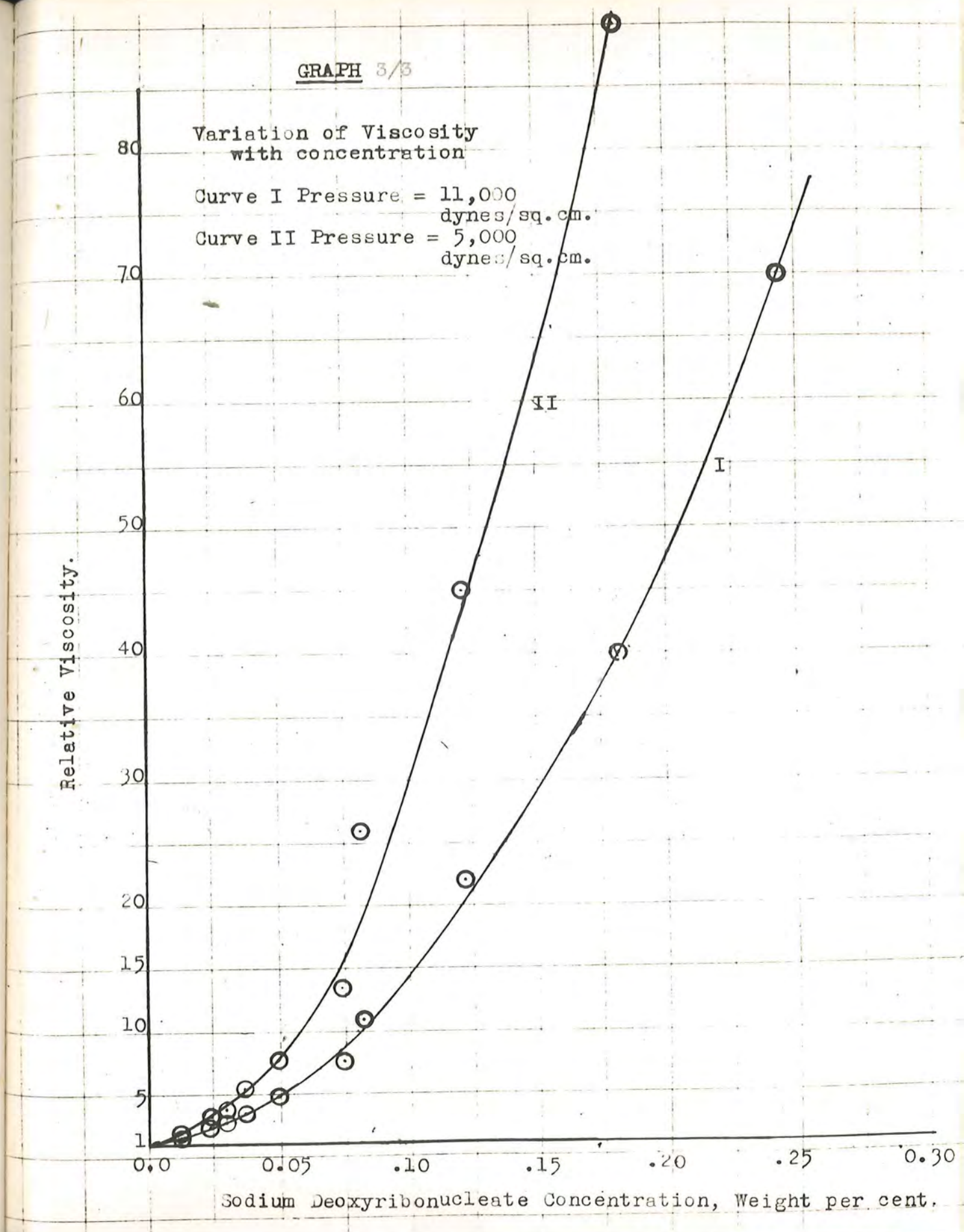


GRAPH 3/2

Structural Viscosities at Different Concentrations. II

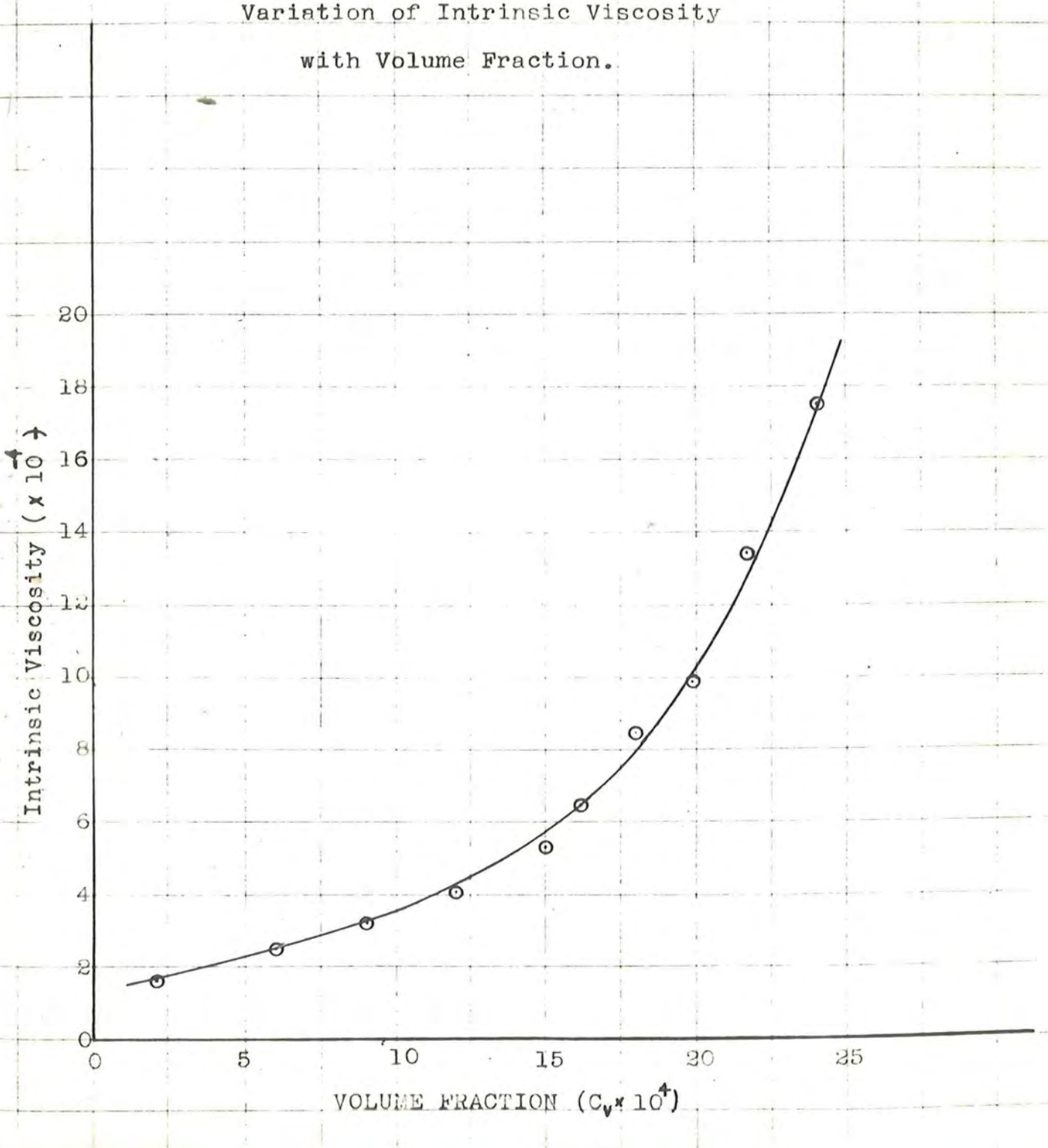
Curve I obtained at c=0.073%.
II c=0.049%.
III c=0.036%.
IV c=0.030%.
V c=0.012%.




GRAPH 3/3
Variation of Viscosity with concentration
Curve I Pressure = 11,000 dynes/sq.cm.
Curve II Pressure = 5,000 dynes/sq.cm.
II
I
Relative Viscosity.
80
70
60
50
40
30
20
15
10
5
1
0.0
0.05
.10
.15
.20
.25
0.30
Sodium Deoxyribonucleate Concentration, Weight per cent.

GRAPH 3/4

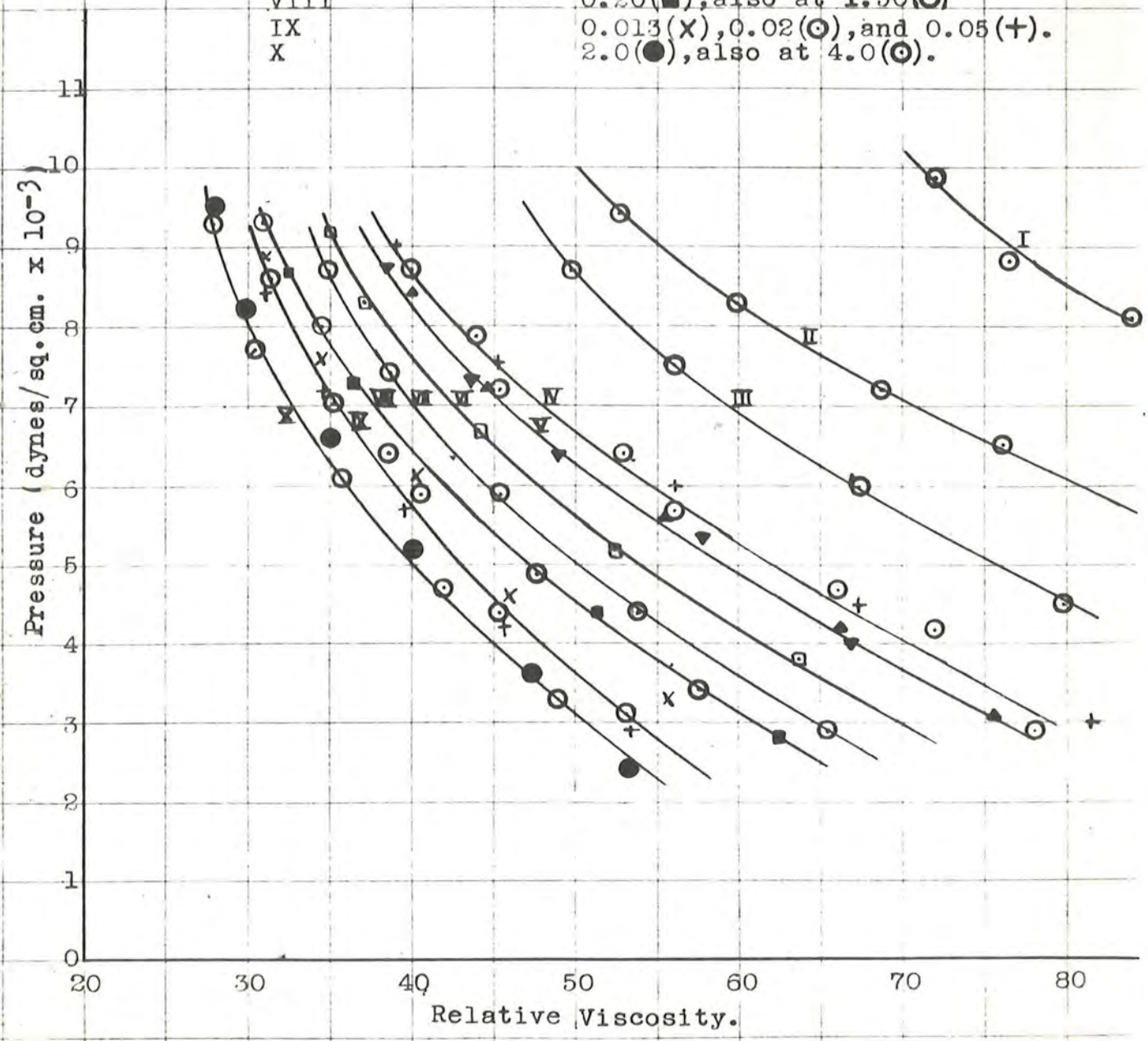
Variation of Intrinsic Viscosity with Volume Fraction.



GRAPH 3/5

Structural Viscosity Curves at different Concentrations of Sodium Chloride.

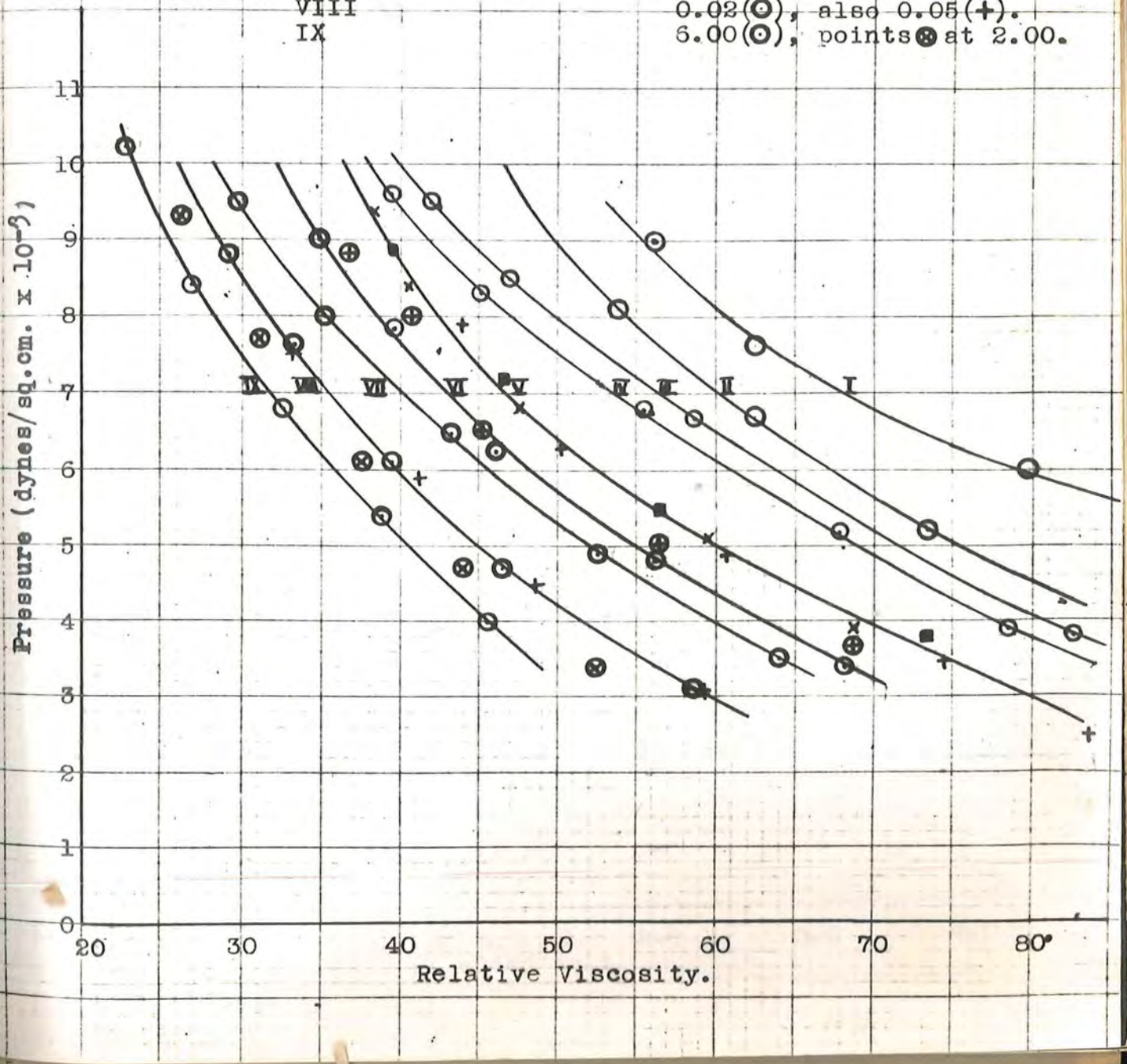
Curve	I	obtained at NaCl=0.001M
	II	0.002
	III	0.0035
	IV	1.00(⊙), also at 0.005(+).
	V	0.50(▲), also at 0.75(▼).
	VI	0.007
	VII	0.01
	VIII	0.20(■), also at 1.50(⊙)
	IX	0.013(X), 0.02(⊙), and 0.05(+).
	X	2.0(●), also at 4.0(⊙).



GRAPH 3/6

Structural Viscosity Curves at different Concentrations of Guanidine Hydrochloride.

Curve I obtained at $C(NH_2)_3Cl$=0.001M
II 0.0025
III 1.00
IV 1.50
V 0.005(+), also 0.40(X)&0.50(■)
Points⊕ obtained at 0.20
VI 0.10
VII 0.01
VIII 0.02(⊙), also 0.05(+).
IX 6.00(⊙), points⊗ at 2.00.

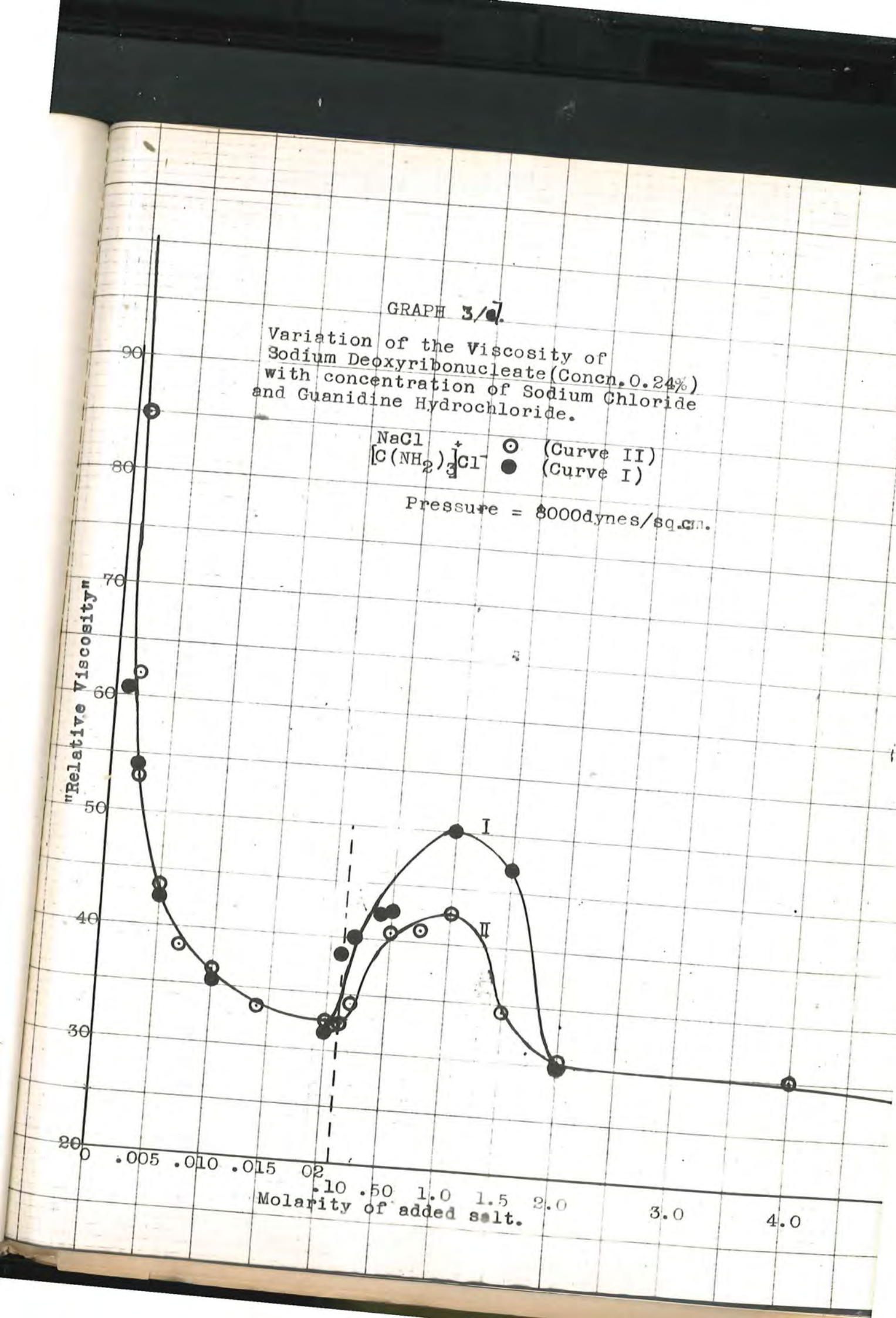


GRAPH 3/d.

Variation of the Viscosity of Sodium Deoxyribonucleate (Concn. 0.24%) with concentration of Sodium Chloride and Guanidine Hydrochloride.

NaCl
$[C(NH_2)_3]^+Cl^-$ ⊙ (Curve II)
● (Curve I)

Pressure = 8000dynes/sq.cm.



GRAPH 3/8

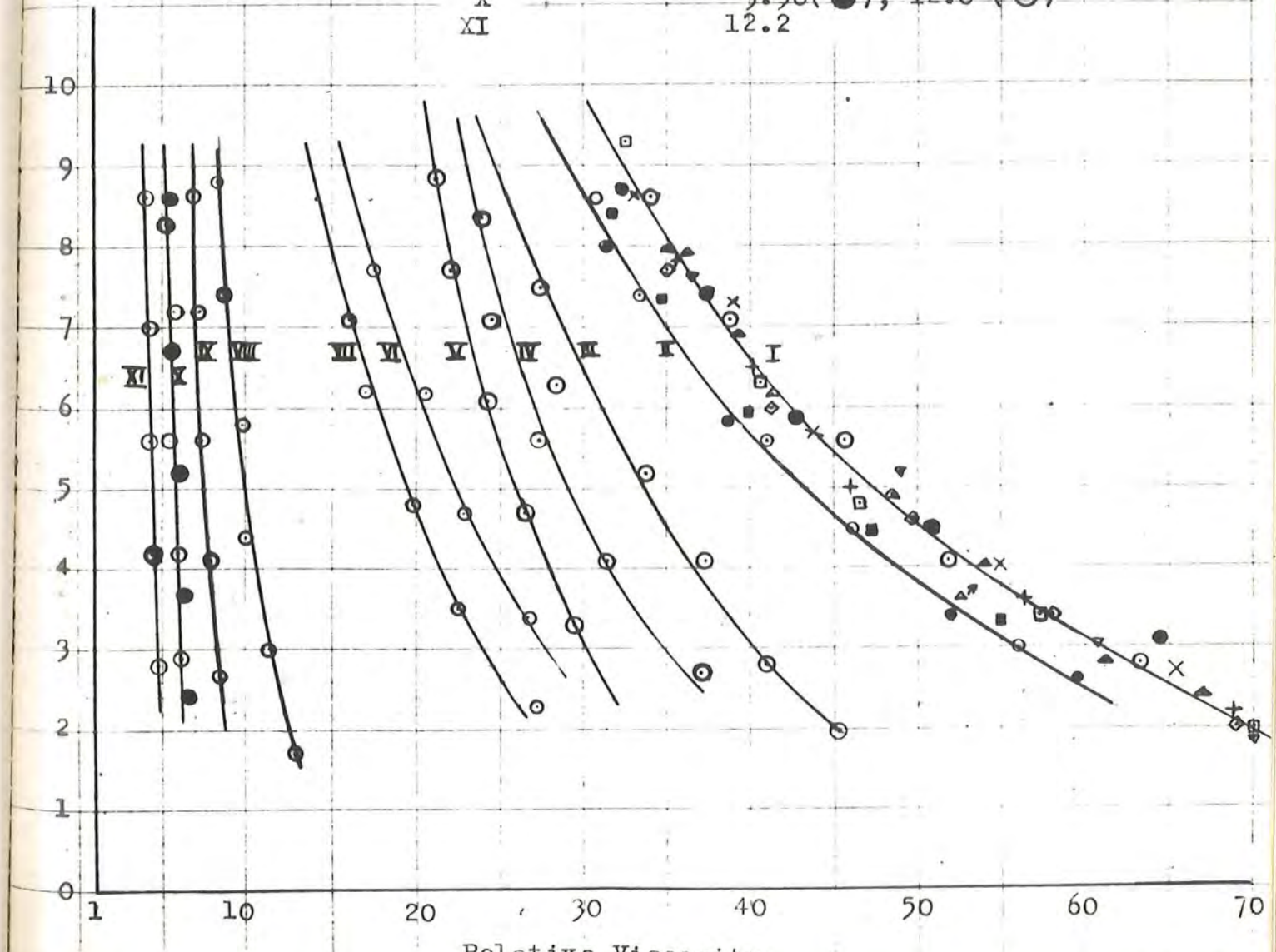
Structural Viscosity Curves at Different pH's.

Curve I obtained at all pH's between 5.30 and 10.6

Points obtained at pH 7.0 ⊙ Points obtained at pH 6.30 X
8.40 ● 6.07 △
9.63 ⊡ 5.40 ▲
10.30 ◈ 5.30 ▽
10.64 +

Curve II obtained at pH 11.1(⊙) and 4.9 (●) Points ■ obtained at pH 10.9 (curve not drawn)

Curve III obtained at pH 4.80
IV 4.74
V 11.8
VI 4.50
VII 4.10
VIII 3.80
IX 3.70
X 3.50(●), 12.0 (⊙)
XI 12.2

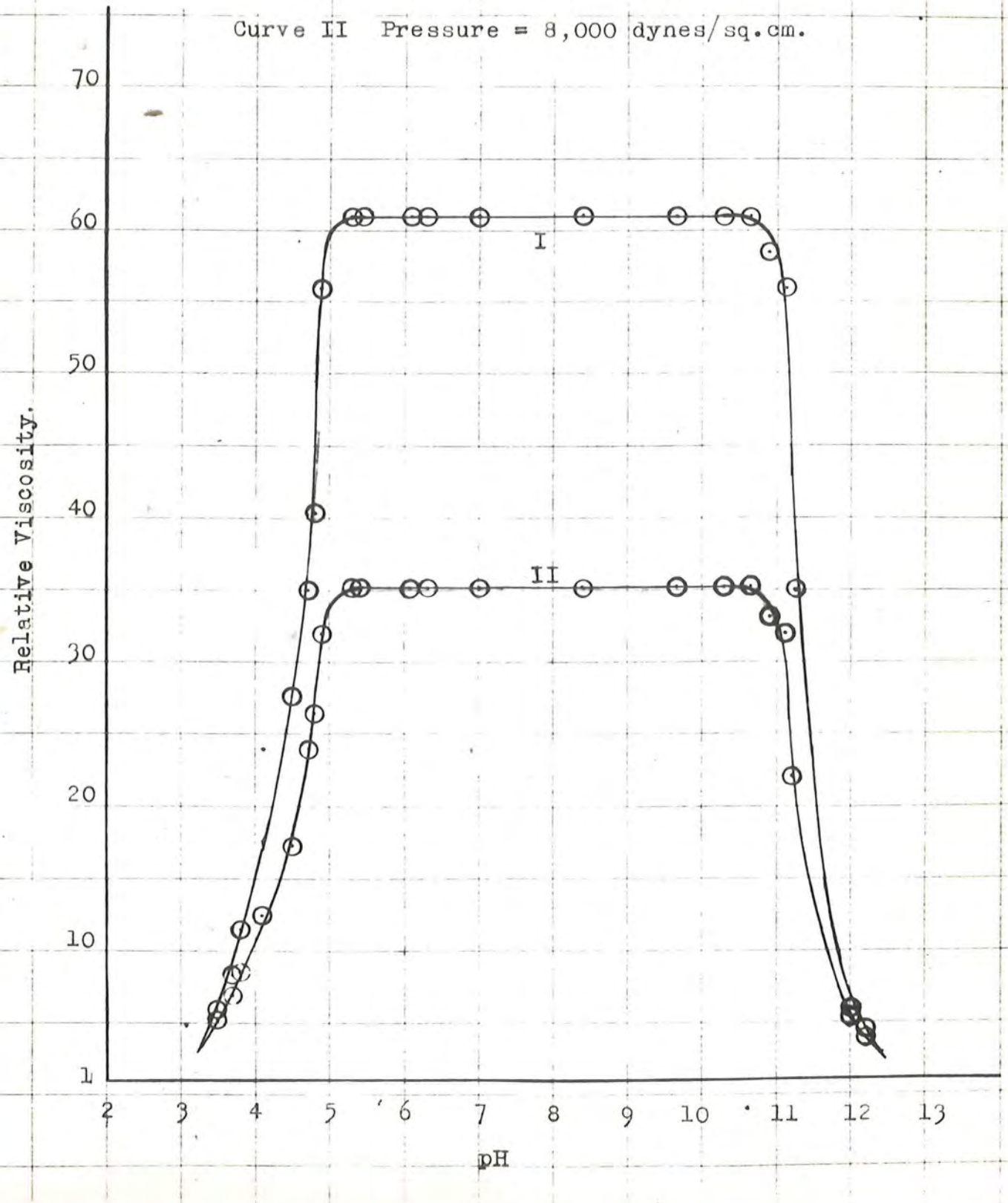


GRAPH. 3/9

Variation of Viscosity with pH.

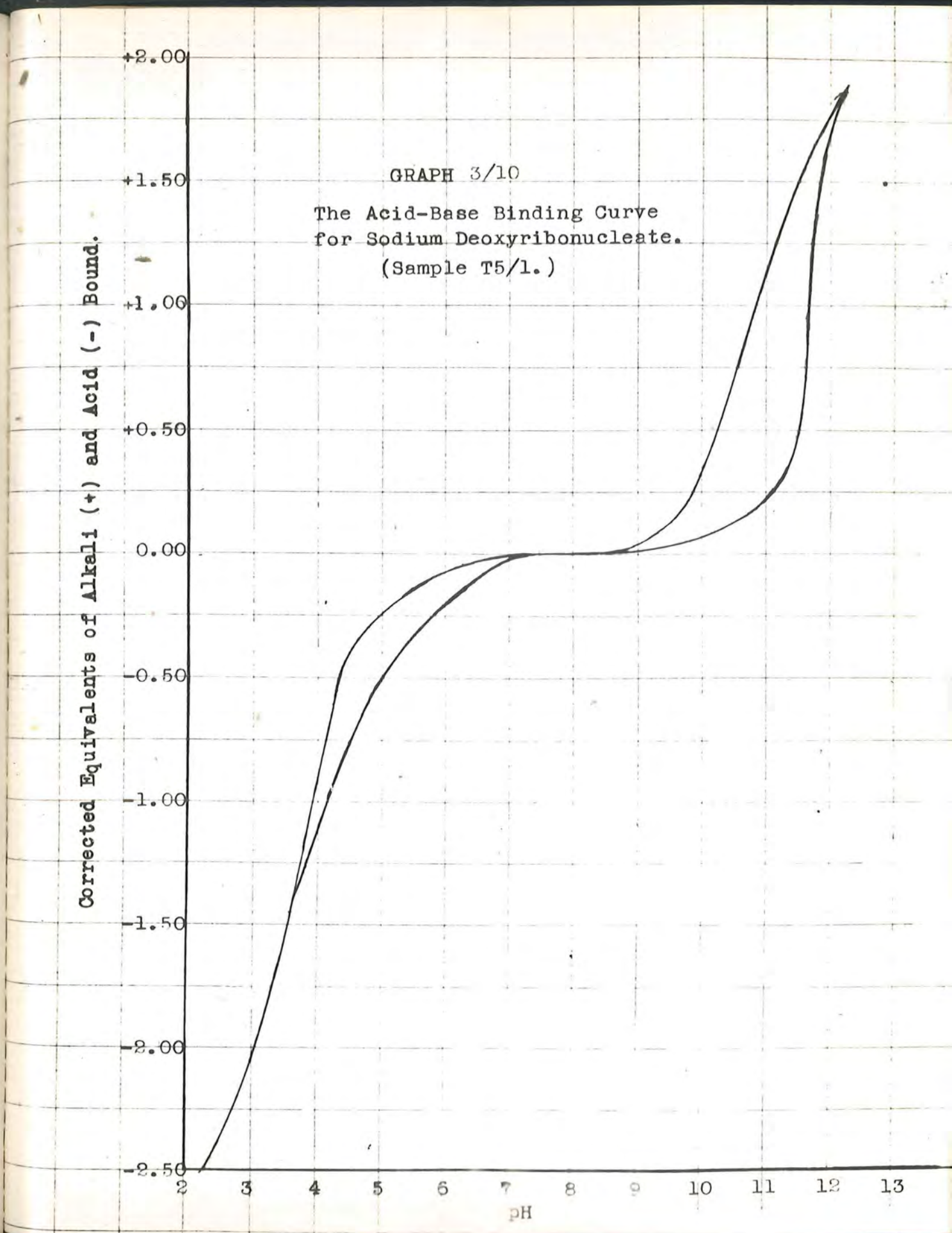
Curve I Pressure = 3,000 dynes/sq.cm.

Curve II Pressure = 8,000 dynes/sq.cm.



GRAPH 3/10

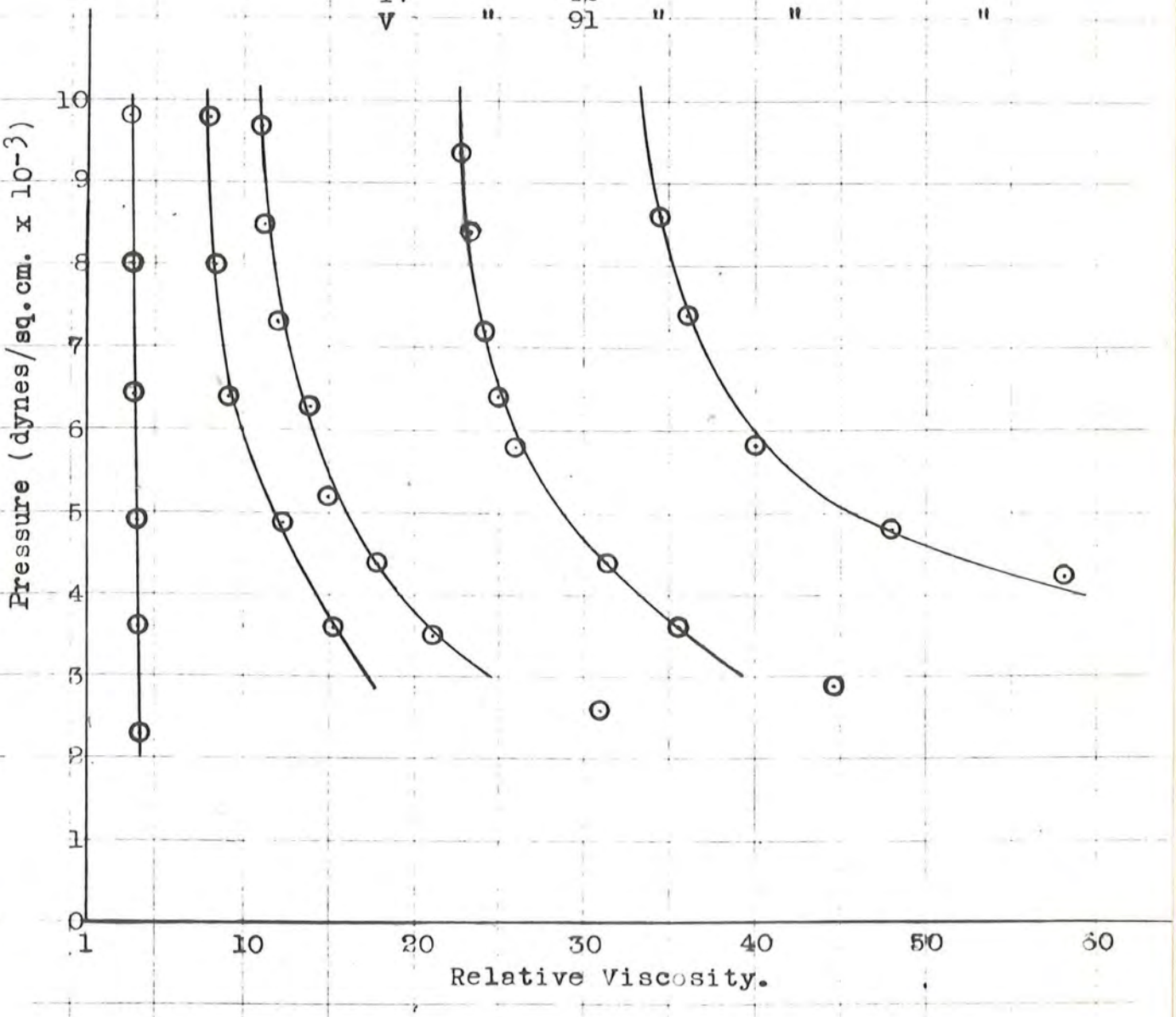
The Acid-Base Binding Curve
for Sodium Deoxyribonucleate.
(Sample T5/1.)



GRAPH 3/11

Structural Viscosity Curves obtained at intervals after neutralisation of an alkali-treated solution of Sodium Deoxyribonucleate.

Curve I obtained 30 minutes after neutralisation.
II " 18 hours " "
III " 24 " " "
IV " 42 " " "
V " 91 " " "

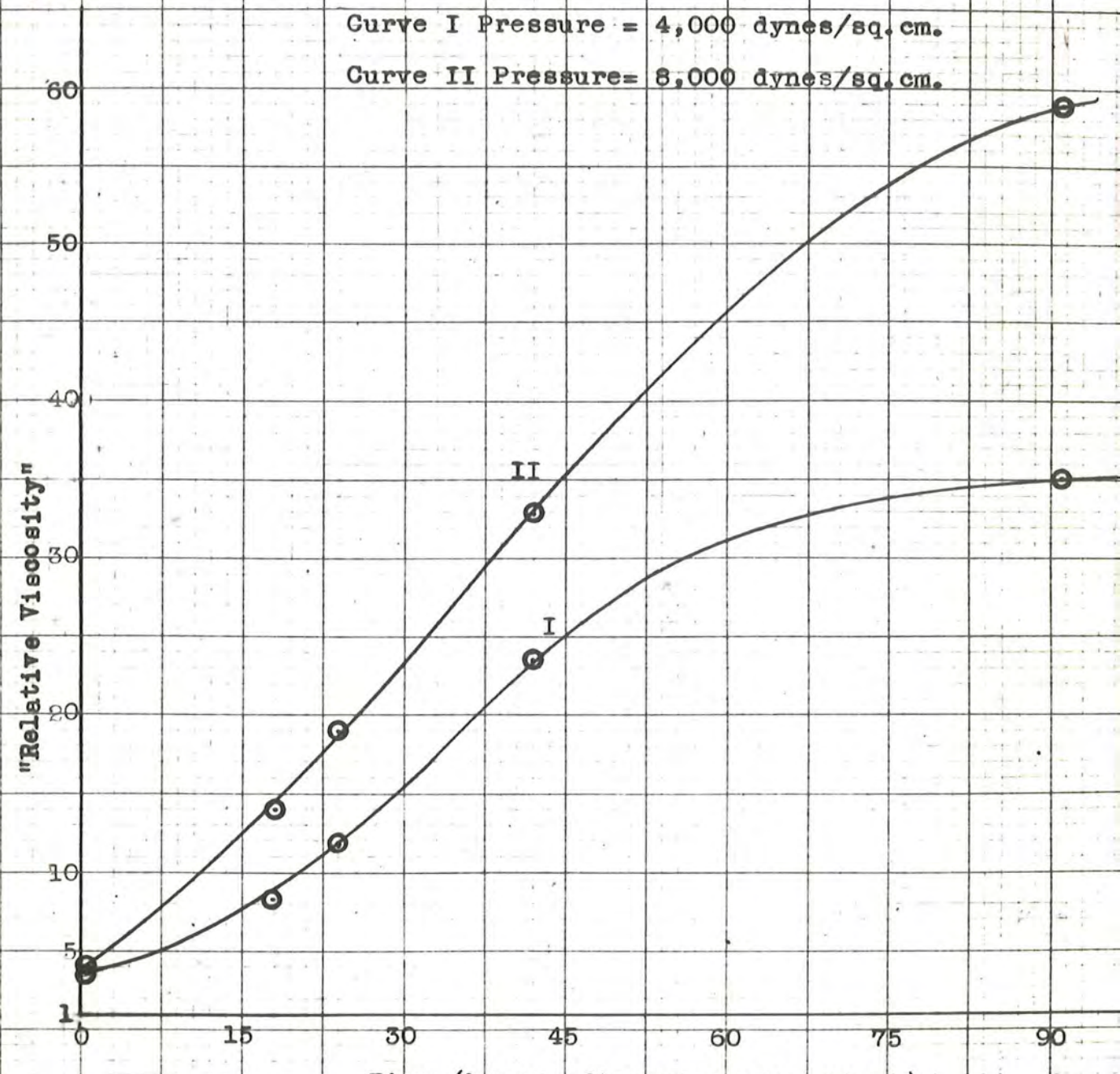


GRAPH 3/12.

Recovery of Viscosity of Sodium Deoxyribonucleate on Neutralisation after alkaline treatment.

Curve I Pressure = 4,000 dynes/sq. cm.

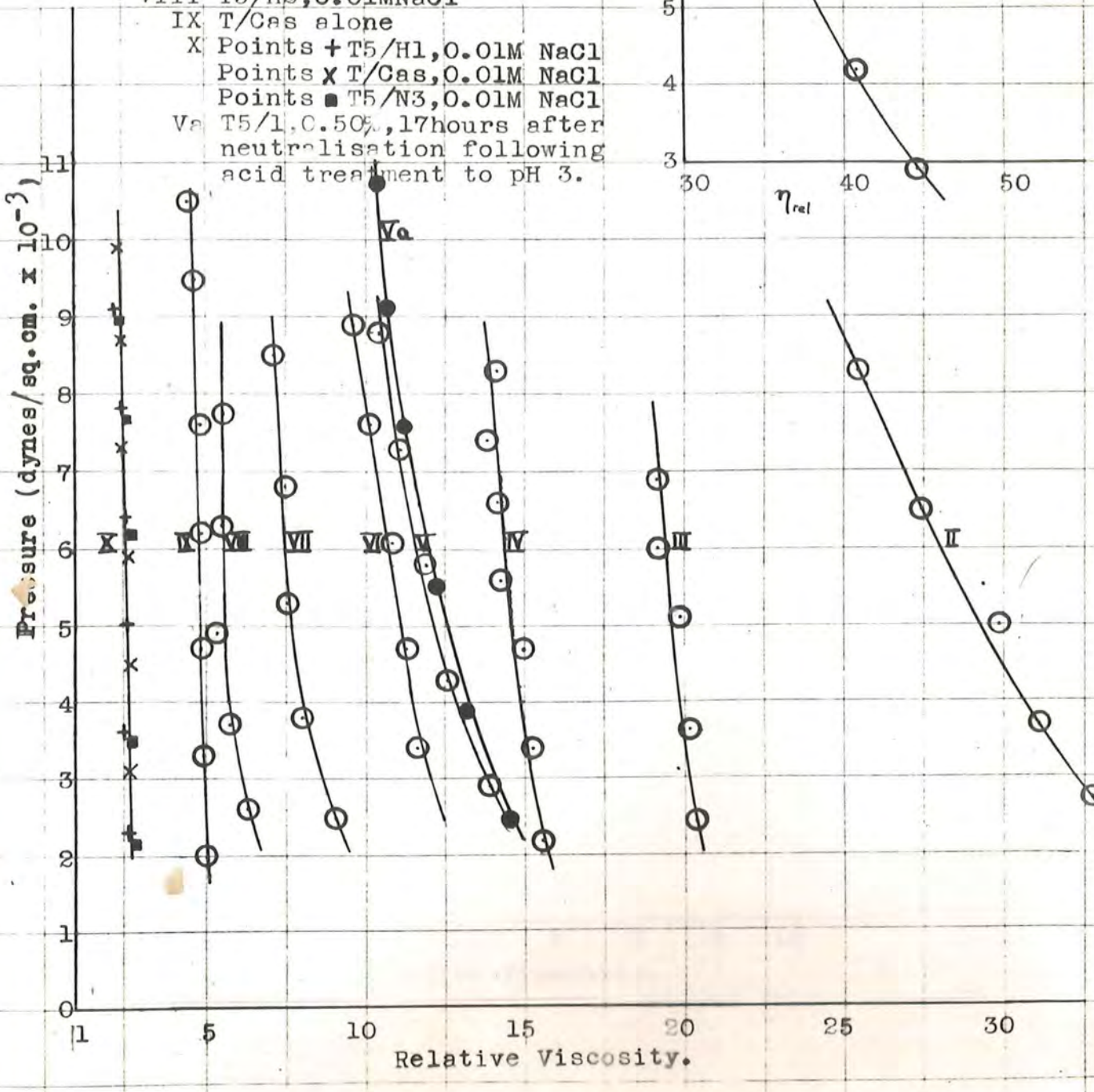
Curve II Pressure= 8,000 dynes/sq. cm.



GRAPH 3/13

Viscosities of Various Samples of Sodium Deoxyribonucleate under different conditions.

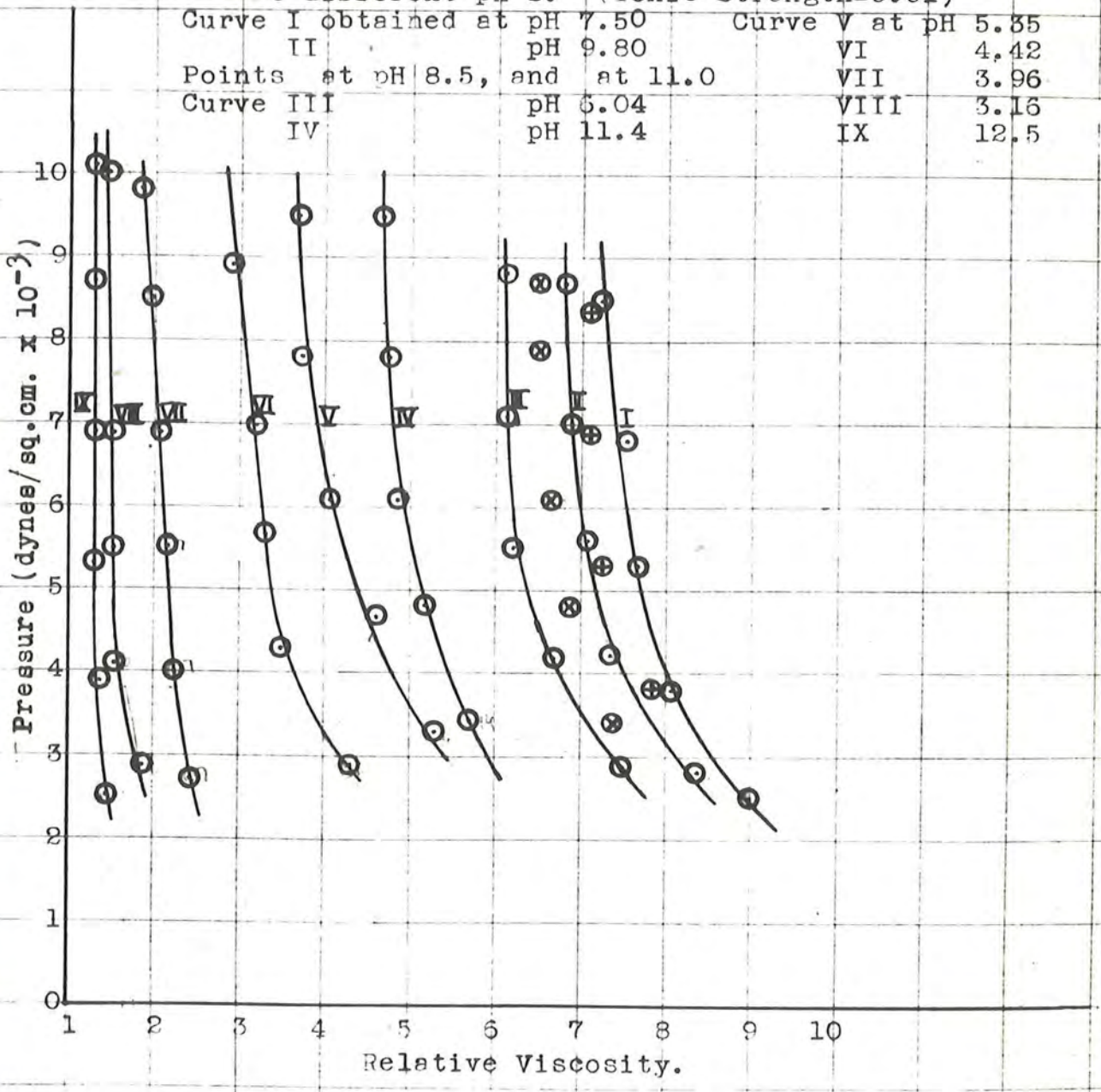
Curve I T5/2 alone
II T5/N1 "
III T5/H2 ,,
IV T5/N3 ,,
V T5/2,0.01M NaCl
VI T5/H1 alone
VII T5/N1,0.01M NaCl
VIII T5/H2,0.01MNaCl
IX T/Cas alone
X Points + T5/H1,0.01M NaCl
Points x T/Cas,0.01M NaCl
Points ■ T5/N3,0.01M NaCl
Va T5/1,0.50%,17hours after neutralisation following acid treatment to pH 3.



GRAPH 3/14

Viscosities of T5/N1 (Concentration 0.24%)
at different pH's. (Ionic Strength=0.01)

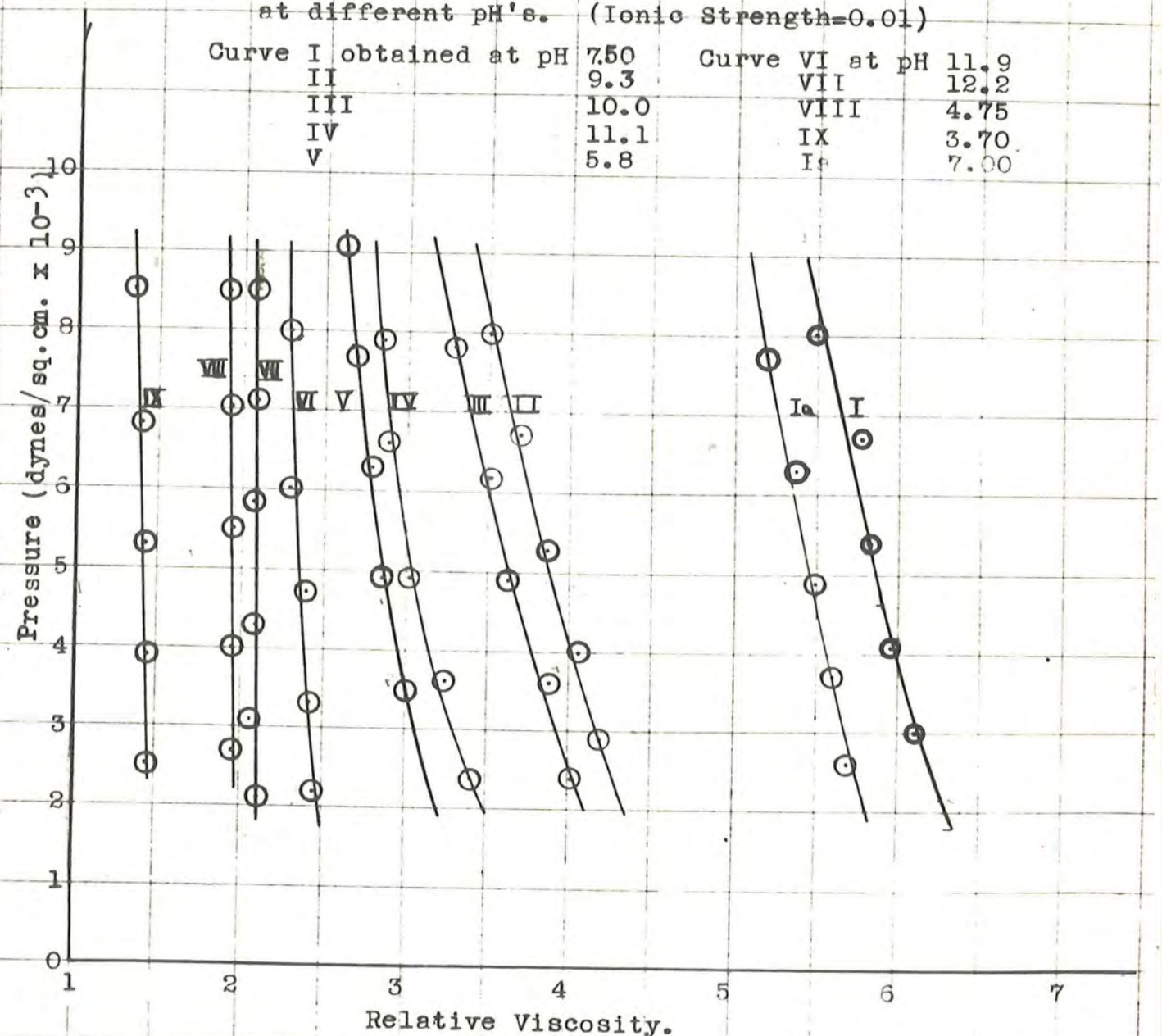
Curve I obtained at pH 7.50
II pH 9.80
Points at pH 8.5, and at 11.0
Curve III pH 6.04
IV pH 11.4
Curve V at pH 5.35
VI 4.42
VII 3.96
VIII 3.16
IX 12.5



GRAPH 3/15.

Viscosities of T5/H2 (Concentration 0.24%)
at different pH's. (Ionic Strength=0.01)

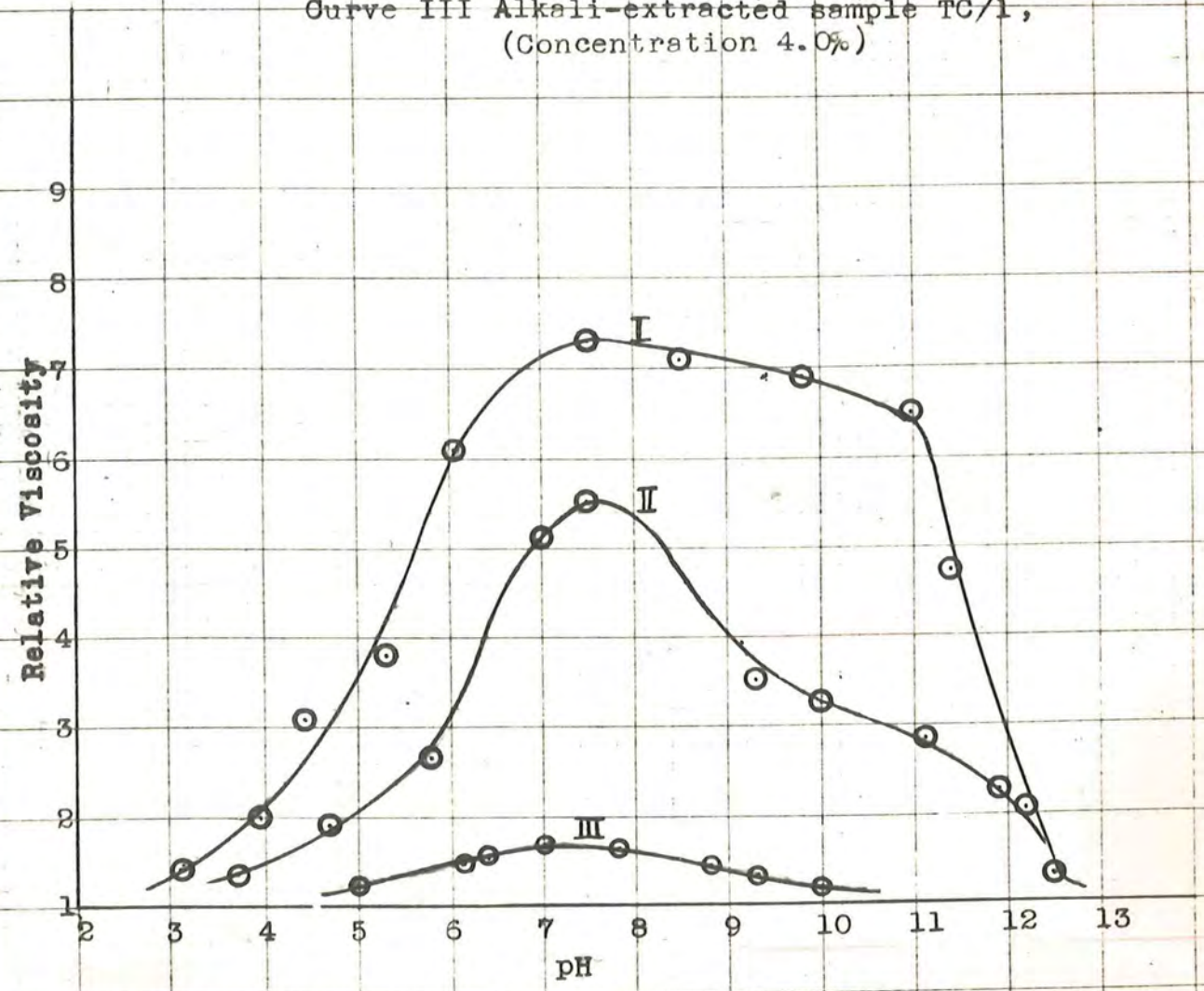
Curve I obtained at pH	7.50	Curve VI at pH	11.9
II	9.3	VII	12.2
III	10.0	VIII	4.75
IV	11.1	IX	3.70
V	5.8	Ia	7.00



GRAPH 3/16

The variation with pH of the viscosity of degraded samples of Sodium Deoxyribonucleate.

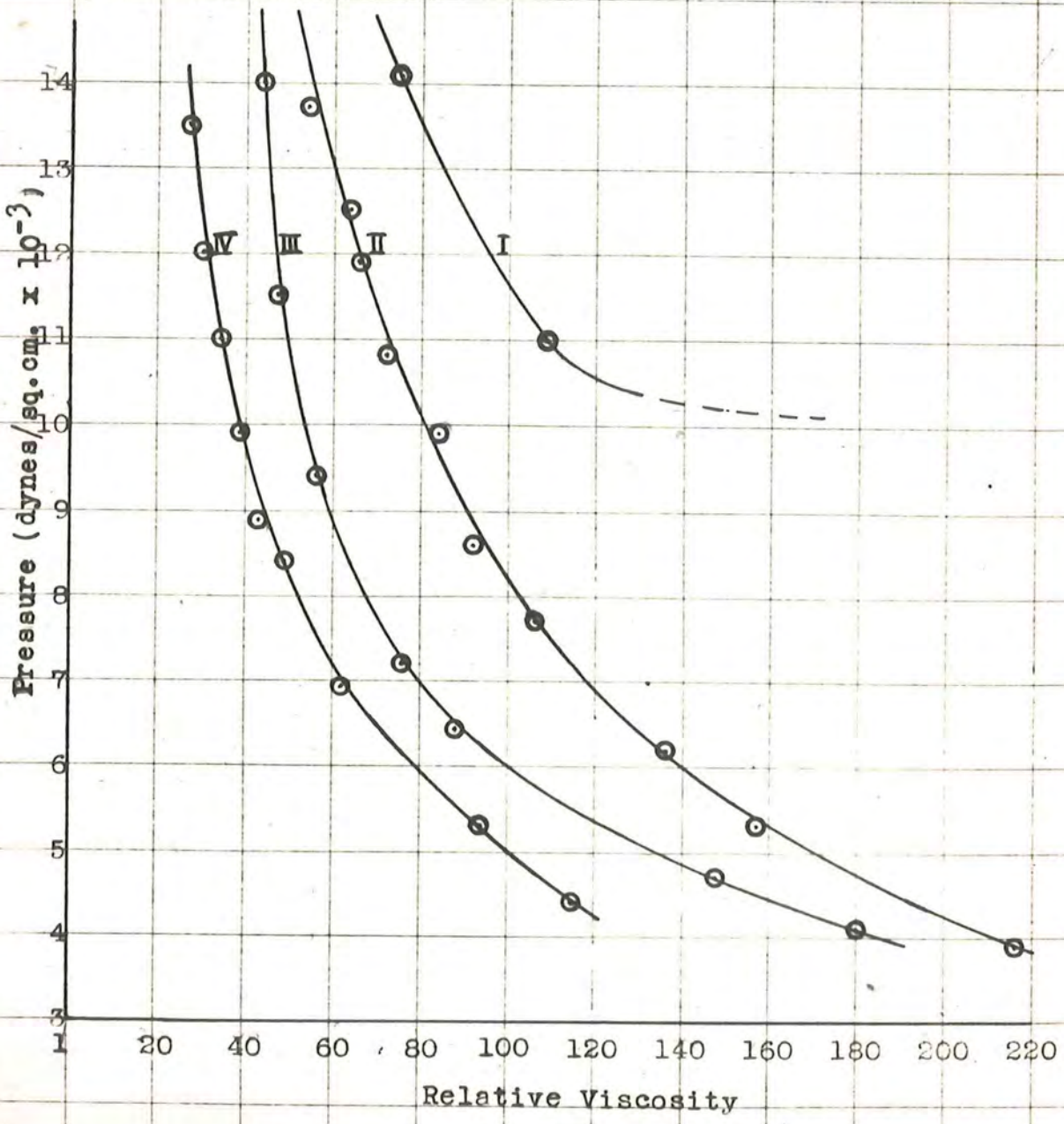
Curve I Alkali-treated sample T5/N1.
Curve II Acid-treated sample T5/H2.
Curve III Alkali-extracted sample TC/1,
(Concentration 4.0%)



GRAPH 3/17

Comparison of the Viscosities
of Sodium Deoxyribonucleate(T5/1)
and its mustard-gas compound(HT/3/53B)

Curve I HT/3/53B, concn. 0.31%
II T5/1, " 0.31%
III HT/3/53B, " 0.21%
IV T5/1, " 0.21%



CHAPTER IV.

Discussion of the Results: The Macromolecular Structure of Sodium Deoxyribonucleate.

The conclusions reached in the previous chapter may be briefly summarised thus: from the rate of variation of viscosity with concentration it is evident that in the conditions of the present investigation, the high viscosities obtained are due primarily to particle interaction and not particle asymmetry, though this factor must play an appreciable part. (This conclusion was partly reached by Greenstein and Jenrette (1): "it is difficult to conceive that this difference (between the viscosities of sodium deoxyribonucleate and the protein myosin) is entirely owing to differences in the magnitude of the axial ratios of the two substances"). For this reason the results obtained in the present investigation have been interpreted qualitatively, and no attempt has been made to apply the hydrodynamical relations postulated for systems of very large very asymmetric particles (See Chap. II). The conclusions of Signer, Caspersson and Hammarsten (2) who applied the equation developed by Burgers to the sodium deoxyribonucleate system, were that the particles acted as long rigid rods of axial ratio about 300. They do not state the viscosity values obtained, or how they were measured; in view of the evidence that their material, extracted by the Hammarsten-Bang process was 'degraded' from the viscosity viewpoint, it is probable that in this case the viscosity increments were primarily due to particle asymmetry. Furthermore, the degree of particle

interaction occurring in the present instance increases very rapidly with concentration of sodium deoxyribonucleate. It is decreased considerably in the presence of low concentrations of neutral salts, but still maintains a fairly high level, even in the presence of nearly saturated salts. The presence of streaming birefringence at all salt concentrations indicates that a high degree of particle asymmetry is maintained. That the drop in viscosity is not caused by 'chemical degradation' of the particles is shown by titration data.

The action of acid and alkali is very different; treatment to pHs outside the stable range completely destroys the capacity for particle interaction, and simultaneously causes a fundamental change in the shape of the micelle, this becoming much less asymmetric, (streaming birefringence data). This change is accompanied by the 'liberation' of the amino and enolic hydroxyl groups of the molecule from an abnormal condition in which they were referred to as being "blocked". It was inferred that these groups were in some way bound up together, as liberation of either acidic or basic groups also caused liberation of the others. In view of the coincidence of the pHs at which this change occurred with the pHs at which the viscosity and streaming birefringence undergo a drastic drop, it must be inferred that the factor causing

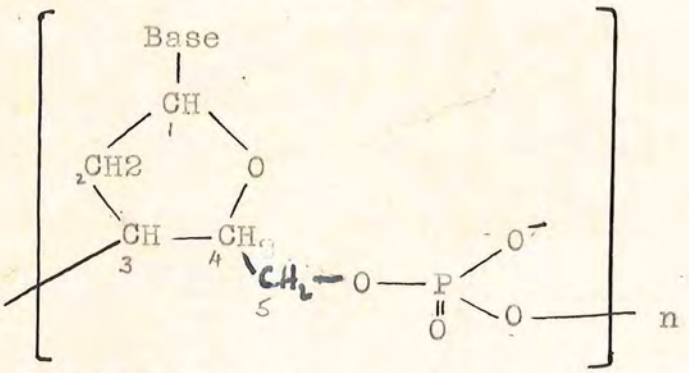
high viscosity, i.e. the macromolecular structure of the deoxyribonucleate ion, is to a great extent determined by the undenatured state of these groups.

The evidence from other sources concerning the macromolecular structure of sodium deoxyribonucleate, when prepared by mild methods, may now be summarised.
The Na:P ratio, together with the evidence (from titration data) that little or no secondary phosphoryl groups are present, shows that the sodium must be largely or completely associated with the primary phosphoryl groups. Therefore this nucleic acid cannot have a branched chain structure involving triply esterified phosphorus, analogous to that postulated for yeast ribonucleic acid by Fletcher Gulland and Jordan (3). The alternative structure, a straight unbranched chain, proposed by Signer, Caspersson and Hammarsten (2) is supported by the X-ray studies (on a similar material) of Astbury and Bell (4) (5), whose conclusions were that the high density (1.63 gm./c.c.) and fibre type of X-ray diagram were compatible with a structure composed of a pile of nucleotides placed flat on top of one another all down the molecular chain. The molecular weights recorded for materials prepared by this (Hammarsten-Bang) process are in accordance with this picture; Signer et al obtained (from viscosity data) values

between 500,000 and 1 million. Ultracentrifugal determinations have given values of 1-2 million (Schmidt et al (6), about 200,000 (Svedberg (7), and 500,000 (Vilbrandt and Tennent (8)).

Further, Astbury (4) has shown that the spacing between the pyrimidine groups in thymic acid (the substance prepared from deoxynucleic acid by quantitative removal of the purines, without other great degradation) is approximately twice as great as the spacing between the nitrogenous groups in the original sodium deoxyribonucleate.

This confirms the view, tentatively advanced by Levene and Simms (9) that the purines alternated with the pyrimidines along the chain; accordingly the weight of evidence is in favour of the polymerised form of Levene tetranucleotide, of structure:



On this basis of the structure of the nucleate ion, the viscosimetric behaviour can be interpreted in the following manner. The particles have regularly recurrent strongly hydrophilic phosphoryl groups all along the chain,

and accordingly the substance is readily soluble in water, in which it must be greatly hydrated. This fact, together with the great asymmetry of the particles, causes in solution a great deal of particle interaction, i.e. the capacity for free movement of the hydrated particles, or micelles, is restricted by their ability to form some type of interlacing network of hydrospheres, the precursor of the gel type of structure. The rigidity of such a loose network increases greatly with increasing concentration of colloid, leading to a greater dependence of measured viscosity on the applied shear force, i.e. to a greater degree of structural character.

The orientation of the particles making up the network by stirring the solution is relatively easy, as the restoring action of Brownian movement is largely inhibited. Accordingly, streaming birefringence is readily shown in dilute solutions, intensified by the highly refringent nature of the nitrogenous groups.

The viscosity of the solution is then determined by the deformability of the network, and the action of neutral salts is thus due to their making the network more readily deformable. This could be accomplished in at least three ways: (a) by a disaggregation of the network, (b) a change in the shape of the individual

micelle, and (c) a change in the structure of the ion atmosphere surrounding the micelle. The latter must certainly occur, and it is possible that the decrease in zeta potential brought about by increasing the ionic strength has an effect on the rigidity of the network. In any case, it is clear that the effect of salts must be largely of an inter-particular nature, as the maintenance of streaming birefringence and the identity of the titration curves of the material in water and concentrated salt solutions prohibits any great change in the constitution of the fundamental particle.

The cause of the maximum in the curve of salt concentration against viscosity at about 1.0 M is obscure, but this behaviour is perhaps analogous to that reported for myosin by Needham and others (10): the viscosity and streaming birefringence of solutions of this protein in the presence of several uniunivalent electrolytes showed maxima between 0.5 and 1.5 M electrolyte concentrations.

This conclusion as to the mode of action of salts contrasts with that originally put forward by Greenstein and Jenrette (1) who assumed that the viscosity drop, relatively greater in the sample they were investigating was due to a depolymerisation of the particles, which they tentatively assumed to be polytetranucleotides held

together by salt-like forces between amino and phosphoryl groups. The colloid osmotic pressures, later investigated by Greenstein (11), of solutions containing sodium deoxyribonucleate and various proteins (which, on a molar basis, are even more effective in decreasing the structural viscosity and streaming birefringence) showed that the nucleate ion did not contribute to the osmotic pressure, however, and accordingly the viscosity drop due to salts and proteins was ascribed to an aggregation of asymmetric particles.

The action of acid and alkali is clearly an effect of different magnitude. The viscosimetric and streaming birefringence behaviour show that not only the capacity of the particles to interact and form the network of long micelles is lost, but also that the asymmetry of the individual micelle is greatly diminished; simultaneously there is a liberation of hitherto blocked amino and hydroxyl groups.

Gulland, Jordan and Taylor (12) concluded that this behaviour could be explained by assuming either (a) that the amino and enolic hydroxyl groups in the native deoxyribonucleate ion were joined by some readily hydrolysable radical, which was rapidly split off at the critical pHs, or (b) that these groups were themselves

held together by the presence of hydrogen bonds.

The assumption (a) has certain limitations. If it were correct, then the postulated radical must (i) be similarly combined with both enolic hydroxyl and amino groups as the "hydrolysis rates" are approximately the same, (ii) have no groups titrating in the pH region 2-12, as no such groups are detectable, and (iii) be present in very low proportion, as experiments on the acid and alkaline treatment of sodium deoxyribonucleate, followed by precipitation after neutralisation gave a nearly-quantitative recovery of the material, and nothing but sodium chloride appeared to be present in the solution after removal of the nucleate. Furthermore, the elementary analyses of both native and acid- or alkali-treated materials were almost identical.

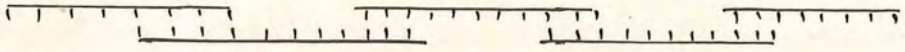
It was therefore concluded that this assumption was unlikely, and that it was very probable that the second explanation was correct, i.e. that the amino and enolic hydroxyl groups of the purine and pyrimidine radicals in the native deoxribonucleate ion were united by hydrogen bonds involving resonance between the forms $-NH_2$ HO- and $-\overset{+}{N}H_3\overset{-}{O}$ - (possibly including water molecules as part of the bond). As Hunter (13) has pointed out, neutralisation (titration) of the acidic and basic groups will tend to break down the hydrogen bonding, which is in accordance with the observed

liberation of groups and the discrepancy between initial and back titration curves.

Although at the present juncture it is undesirable to speculate further as to the macromolecular state of this nucleic acid, it will be shown that this explanation of its acid-base behaviour also relates the viscosimetric and streaming birefringence phenomena. Moreover, bonding of this type is most probably of great importance in determining the unique structure of undenatured proteins (for example, see Wu (14)), and Mirsky and Pauling (15) have postulated that protein denaturation is due to the breaking of these bonds with consequent loss of unique ordered structure. The results obtained with sodium deoxyribonucleate in this investigation show certain resemblances to those recorded for egg albumen; Crammer and Neuberger (16), by absorption spectra measurements, confirmed the observations of Cannan, Kibrick and Palmer (17), from titration data, that the phenolic groups in native egg albumen were blocked; furthermore, denaturation of the protein by acid, alkali, alcohol or ultraviolet light led to the liberation of the phenolic groups in their normal titratable state. This behaviour was ascribed by Crammer and Neuberger to hydrogen bonding between the phenolic hydroxyl groups and an adjacent carboxylate ion. In this connection, the observations of Hollaender, Greenstein and Jenrette (18) on the action of ultraviolet light on

sodium deoxyribonucleate solutions are of interest. The viscosity and streaming birefringence dropped steadily with exposure to ultraviolet light, and after a short time were found to be little greater than that of the solvent. The phenomenon was irreversible, and was ascribed by these authors to the slow depolymerisation of the macro-particles to smaller less asymmetric ones, by analogy with the effect of salts.

The viscosimetric behaviour at the critical 'denaturation' pHs can accordingly be interpreted on this basis. It must be inferred from the experimental results that the hydrogen bonding between the amino and hydroxyl groups is instrumental in maintaining the particles in their great asymmetry. This could be achieved in either of two ways: (a) the individual polynucleotide chains are not themselves markedly asymmetric, but the great length of the micelle is due to the formation of a polymerised polynucleotide, in which the constituent chains are united down their common length by hydrogen bonding between the amino groups of one chain and the hydroxyl groups of the other, and vice versa, as indicated in the diagram. The possible number of such bonds, up to a maximum of two per four phosphorus atoms, would lead to the formation of a very rigid ultimate particle, whose length could be very great.



(The formation of a very strong link between two chains which possess oppositely polar groups at regularly recurrent intervals down the length was discussed by Astbury (5) in the case of the nucleic acid-protein relationship in the chromosomes. He demonstrated by X ray analysis that the spacing between successive phosphorus atoms in the nucleate ion was the same (3.34 A) as the spacing between side chains of an extended polypeptide chain).

The action of acids and alkalis on this model is to sever the hydrogen bonds uniting the individual polynucleotide chains, which are thus liberated; being relatively small and flexible they do not interact to form the network characteristic of the micellar state, and the solution is not very viscous. Similarly the destruction of the very asymmetric particles causes loss of streaming birefringence.

(b) The alternative explanation of the action of the hydrogen bonds in lending rigidity to the micelle is that the individual polynucleotide chain is itself very long and asymmetric, but is only held rigidly linear by hydrogen bonding between the amino and hydroxyl groups of adjacent radicals in the same chain. The action of acids and alkalis destroys the hydrogen bonds holding the chain rigid; the latter is then flexible and may rapidly coil up, forming a particle which is not markedly asymmetric, though of the same mass as before.

Such a change in the shape of the micelle would have an effect on the viscosity and streaming birefringence similar to that produced by the break up of the polymerised polynucleotide chain postulated above.

In this connection, the behaviour of the product obtained by the action of mustard gas on sodium deoxyribonucleate is of great interest. Solutions of this substance show very high viscosity values, varying greatly with the pressure, at acid pHs well below the critical for the original material (Graph 3/17). As stated earlier, the evidence from titration data showed the presence of approximately two blocked groups for every 'H-residue', with the inference that each residue was linked to two groups. This could be accomplished by cross-linking between chains, or by attachment to two suitably spaced groups in the same chain. In either case, the presence of high viscosity of acid solutions of this substance strongly supports the view that the viscosity of native, sodium deoxyribonucleate solutions is due to some similar 'bridge formation' whereby the particles are kept rigid and asymmetric.

The above explanation of the effect of acid and alkali upon the macromolecular structure of sodium deoxyribonucleate agrees in some respects with that put forward by Vilbrandt and Tennent (19) who assumed that fission occurred at random

intervals down the chain, leading to the formation of a polydisperse system of smaller particles. They do not quote molecular weight values in acid or alkaline solutions, and they did not speculate as to the nature of the linkage that was broken; the titration evidence of Gulland, Jordan and Taylor (12) show that such 'hydrolysis' cannot involve internucleotide phosphoester bonds. The neutralised solution was shown to be polydisperse with regard to particle size, some molecules being larger than those of the monodisperse original. It was suggested that the property of gel-formation was due to the 'binding action' of these molecules.

The reversibility of the change in macromolecular structure brought about by acid and alkali is clearly limited, if the viscosity streaming birefringence and titration data of Chap.III may be taken as criteria. Thus the viscosimetric behaviour of solutions neutralised after acid or alkaline treatment was similar, in that there was a gradual but incomplete recovery of the capacity of the particles to interact, without a restoration of the great asymmetry of the original micelle. (Streaming birefringence was in no case detectable). The greater gel-like structure developed in these solutions was especially shown in those neutralised after acid treatment. The original type of hydrogen bonding between amino and hydroxyl groups was never recovered. (titration data).

The viscosity values of the substances precipitated after neutralisation were in general greater than those of the solutions before precipitation, and moreover some degree of hydrogen bonding was shown by the hydroxyl groups (very little or none was shown by the amino groups).

The behaviour of those samples which had stood for four days before precipitation reproduced on a smaller scale the characteristics of the native substance with regard to variation of viscosity with pH, an indication that some degree of the original macromolecular structure had been regained. The viscosity values for these materials were similar to those recorded by Vilbrandt and Tennent (19) for a sample prepared by the Hammarsten-Bang process, and also to those obtained by the author for a material prepared by this process, ("T/Cas"), and for another material (T5/2) prepared by a slightly acid extraction.

On the basis of this evidence it is possible to speculate on the causes of the differences in behaviour shown by (a) the original solution of sodium deoxyribonucleate, (b) the solutions neutralised after acid and alkaline treatment, and (c) the solutions of the precipitated samples.

The increase of viscosity with lapse of time shown by (b) cannot be due to the reformation, in part, of the original structure and it is possible that the difference between this and the solution of the original material lies

mainly in the availability of the amino and hydroxyl groups, i.e. it is possible that in the original these groups are combined with each other and are out of contact with the aqueous phase, whereas in the neutralised solutions, the groups, though still attracting one another (possibly by hydrogen bonding through water molecules) are part of a continuous aqueous phase, and so titrate normally.

The sudden development of hydrogen bonding of the hydroxyl groups on precipitation with alcohol (i.e. removal of all water), when this was not present immediately before precipitation, lends some support to this hypothesis, which is also in accordance with the viscosimetric behaviour of the materials, and with the conclusions of Vilbrandt and Tennent. It should be pointed out that their observations concerning the inhomogeneity of the substance after alkaline treatment, although supporting the possibility of the gradual re-uniting of the small polynucleotide chains postulated above, does not preclude the alternative possibility of the slow 'unkinking' of coiled up particles; the differing sedimentation constants shown would be then due to the presence of molecules in varying states of compactness, and hence differing frictional constants.

In summary, therefore, the results of the observations of viscosity, streaming birefringence, and electrometric titration data, in conformity with the results of other

investigators, lead to the conclusion that the macromolecular structure of sodium deoxyribonucleate isolated by mild methods is that of a very long, very asymmetric rigid particle. Further, it has been shown that this condition is in all probability due to the presence of hydrogen bonds, in a manner possibly analogous to that responsible for the unique structures of undenatured proteins. The hydrogen bonds, which may link groups either in the same, or in different, chains, may be severed by theaction of acid or alkali, when the original structure is completely destroyed. On neutralisation after such treatment, some slight degree of recovery of the initial structure is indicated, but this can never go to completion.

The implication of this irreversible change, and the fact that great variations have been recorded for the physical properties of materials isolated by different methods is clearly that either the deoxyribonucleate in the nuclei of the cells is present in a very highly ordered state of hydrogen bonding, or that this is produced during the isolation process. The latter alternative would appear to be unlikely.

Although in themselves the presence of blocked groups and the occurrence of high viscosity values are not evidence that a material closely resembles the original nuclear substance, the fact that the destruction of these properties is an irreversible process does strongly suggest, as above, that they were present in the nucleoprotein.

It is therefore very probable that the material used in the present investigation, having these properties to a greater extent than any hitherto examined, approximated more closely to the native substance than the latter; in this case, the mild action of acid and alkali can with propriety be termed a degradative process.

Furthermore, the similarity in behaviour of the products of mild degradation with those of substances isolated by the Hammarsten-Bang process tends to show that this method leads to the isolation of a more or less degraded form, i.e. that at some stage in the extraction, an alteration has occurred in the hydrogen bond structure of the substance (The use of acidic or alkaline processes of extraction is clearly even more likely to lead to a degraded product). Moreover, since the Hammarsten-Bang process differs only slightly from that of Gulland, Jordan and Threlfall (20) it is very probable that the material used in the present investigation had undergone some (probably slight) degree of degradation, or denaturation.

Since, therefore, it is probable that the amino and hydroxyl groups of the nucleic acid part of the nucleoprotein are bound together, the link between acid and protein is most likely to involve the phosphoryl groups, in conformity with the present generally accepted view. Since the extraction process must break this link, and it has been shown that this can be accomplished by mild methods, the question is raised as to the relationship between nucleic acid, protein and nucleoprotein.

This relationship has been widely investigated (21,22, 23,24), and it seems clear, especially from the work of Greenstein (23) that the denaturation of the protein part of the nucleoprotein is an essential step in the liberation of the nucleate ion. The problem may therefore be preliminarily divided into two stages: (i) extraction of a true native nucleoprotein, perforce by methods not involving denaturing agents for either nucleic acid or protein, and (ii) denaturation of the protein part without any change in the macromolecular structure of the nucleate part.

It is evident that the alcohol precipitation stage in the extraction of the nucleoprotein described in Chapter III is likely to denature the protein part; this was found to be the case, the "nucleoprotein" being incompletely soluble in water, leaving a white protein precipitate: further precipitations

gave products progressively richer in nucleic acid.

It appeared probable that the methods of electrophoretic separation would be very useful in the investigation of this problem; as a prelude to this, the electrophoretic properties of the deoxyribonucleate ion were investigated under various conditions. The results obtained, and the conclusions to be derived from them, form the subject matter of the remainder of this thesis.

REFERENCES TO CHAPTER IV

1. Greenstein and Jenrette, J.Nat.Cancer Inst. 1940, 1, 77.
2. Signer, Caspersson and Hammarsten, Nature 1938, 141, 122.
3. Fletcher, Gulland and Jordan, J.C.S. 1944, 33.
4. Astbury and Bell, Nature 1938, 141, 747.
5. Astbury, Symposia Soc.Exp.Biol. 1946, 1, 66.
6. Schmidt, Pickels and Levene. J.Biol.Chem. 1939, 127,251.
7. Svedberg, in The Ultracentrifuge, Oxford 1940, p.443.
8. Tennent and Vilbrandt, J.Amer.Chem.Soc. 1943, 65
9. Levene and Simms, J.Biol.Chem., 1925, 65, 519.
10. Needham, Kleinzeller, Miall, Dainty, Needham and Lawrence, Nature 1942, 150, 46.
11. Greenstein, J.Biol.Chem. 1943, 150, 107.
12. Gulland, Jordan and Taylor, J.C.S. 1947, 1131.
13. Hunter, Chem. and Ind. 1944, p.155.
14. Wu, Chinese Journal of Physiology 1931, 5, 321.
15. Mirsky and Pauling, Proc.Nat.Acad.Sci.Wash. 1936, 22, 439.
16. Crammer and Neuberger, Biochem.J. 1943, 37, 302.
17. Cannan, Kibrick and Palmer, Ann.N.Y.Acad.Sci. 1941,41,243.
18. Hollaender, Greenstein and Jenrette, J.Nat.Cancer Inst. 1941, 2, 23.
19. Vilbrandt and Tennent, J.Amer.Chem.Soc. 1943, 65, 1804.
20. Gulland, Jordan and Threlfall, J.C.S. 1947, 1129.

21. Carter and Hall, J.Amer.Chem.Soc. 1940, 62, 1194.

22. Mirsky and Pollister, Proc.Nat.Acad.Sci. U.S.A. 1942, 28, 344.

23. Greenstein and Jenrette, J.Nat.Cancer.Inst. 1941, 1, 845; 2, 301.

24. Cohen, J.Biol.Chem. 1945, 158, 255.

CHAPTER V

The Electrophoresis of Bio-Colloids.

Section (i) Introduction

(ii) General theory of the moving boundary.

(iii) The effects of conductivity changes at the boundary; The visible boundary anomalies.

(iv) Other sources of error.

(v) The modified Tiselius Electrophoresis Apparatus.

(vi) Summary.

Section (i)

Introduction.

The electrokinetic properties of a colloidal material in solution are determined by the interaction between the particles of the substance and the molecules of the solvent (the dispersion medium), and they may be studied by observation of either of the twin phenomena of electro-osmosis or electrophoresis.

In the first case, the substance must be insoluble, and the motion, under the influence of an applied electric field, of the liquid in contact with it, is studied. Systems which are fibrous, or have small pores may be studied in this way.

In electrophoresis, the motion of the colloidal particles through the resting dispersion medium is studied, either by direct observation of a single particle (micro method), or by observation of the migration of the whole colloidal solution. The latter may be accomplished by measuring the concentration changes occurring on passing a current, or by observation of the motion of the boundary formed between the colloidal solution and the solvent, i.e. the moving boundary method.

The moving boundary method is of general application to water soluble colloidal mixtures, and is of special use

for the investigation of possibly complex substances of biological origin.

The method was first devised by Lodge, and was used by him to measure the mobilities of inorganic ions; in 1897, Picton and Linder (1) studied the motion of colloidal particles by a similar method. Subsequently, many investigations were performed with colloids of inorganic and organic origin, but owing to a lack of understanding of the necessary precautions, the results obtained were unreliable, and the method was severely criticised (2,3).

In 1930, Tiselius (4) in a most comprehensive treatment, placed the whole subject on a secure footing, formulating rules whereby the errors inherent in the method could be minimised, and proving his conclusions by rigorous experimentation.

The subsequent advances in protein chemistry, made possible by the use of this technique, must be considered to be due in no small part to his investigations.

Further theoretical contributions have been made by Henry and Brittain (5), Dole (6), and Svensson (7), and the original apparatus designed by Tiselius has been considerably modified (notably by Tiselius (8), Longsworth (9) Philpott (10) and Svensson (7)) following the greatly increased use of the method, and there is now available an apparatus in which considerable power may be applied, and in

which the refractive index gradients in the boundaries may be directly observed with great precision.

Accordingly, every component of a complex colloidal mixture may be detected and estimated, and, if desired, isolated by a purely physical process. The ability to carry out such a process is of immense value in the varied fields of biochemistry, where so many of the materials are extremely sensitive to chemical reagents.

In addition, it is possible to determine accurately the characteristic electrokinetic properties of water soluble colloids, and this has been the main purpose during the present investigation, in which the electrophoretic behaviour of sodium deoxyribonucleate has been studied under various conditions.

Those aspects of the theory of electric migration in liquids which are relevant will now be considered.

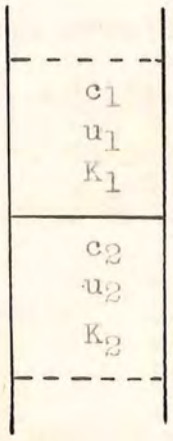
Section (ii). General Theory.

(a) The equation of the moving boundary.

The fundamental equations governing the motion of a boundary due to an applied electric field were given originally by Kohlrausch (11) and almost simultaneously by Weber (12); the further conditions which must be observed if true mobilities are to be calculated have been summarised by

Svensson (7). The following treatment has been mainly derived from the comprehensive work of Svensson.

Taking an arbitrary case in which a boundary is formed between two different concentrations c_1 and c_2 of an ion, and representing the conductivity as K_1 and the mobility of the ion as u_1 in the zone of concentration c_1 and correspondingly K_2 and u_2 in the zone of concentration c_2, as in the diagram, then on passing a current i, the amounts of the ion which pass the sections A and C (infinitely distant from the electrodes) in the figure will be:-



Past the section A: $\left(\frac{c_1 u_1 \ i}{K_1}\right)$,

and

Past the section B: $\left(\frac{c_2 u_2 \ i}{K_2}\right)$,

per unit time and cross sectional area.

(The concentrations are in terms of electrochemical equivalents, with the sign of the charges)

Hence the quantity $\left(\frac{c_1 u_1 i}{K_1} - \frac{c_2 u_2 i}{K_2}\right)$ represents the rate of increase of the amount of the ion per unit cross sectional area.

The boundary moves at a velocity V, and accordingly the rate of gain of the ion will be $\pm V(c_1 - c_2)$, i.e.

$$\frac{c_1 u_1 i}{K_1} - \frac{c_2 u_2 i}{K_2} = V(c_1 - c_2) \qquad (1)$$

Since the conductivities K_1 and K_2 are not, in general, equal, the boundary possesses two mobility values U_1 and U_2, given by:

$$V = \frac{U_1 i}{K_1} = \frac{U_2 i}{K_2} \qquad (2)$$

$$\text{i.e. } U_1 K_2 = U_2 K_1 \qquad (3)$$

Hence equation (1) may be rewritten with the aid of equations (2) and (3) in the symmetrical form:

$$\frac{c_1 u_1 i}{K_1} - \frac{c_2 u_2 i}{K_2} = \frac{U_1 c_1 i}{K_1} - \frac{U_2 c_2 i}{K_2}$$

$$\text{i.e. } \frac{c_1(u_1 - U_1)}{K_1} = \frac{c_2(u_2 - U_2)}{K_2} \qquad (4)$$

The unsymmetrical form

$$(c_1 - c_2)(u_1 - U_1) = \left(\frac{K_1}{K_2} - \frac{u_1}{u_2}\right) c_2 u_2 \qquad (5)$$

may also be derived.

These equations must be satisfied by every ion present on one or both sides of a boundary. The implications of this requirement will now be considered.

(b) Determination of True Mobility.

For the purpose of determining the true mobility of the ion under investigation, it must be claimed that the mobility

of the boundary represents the mobility of the ion, i.e. that $u_1 = U_1$ or $u_2 = U_2$ for the ion in question.

Since equation (3) is symmetrical, only one of these cases need be considered.

If the mobility of the boundary as calculated from the conductivity K_2 of the lower solution is equal to the mobility of the ion at the concentration c_2, i.e. if $U_2 = u_2$, then the right hand side of equation (4) becomes zero, and accordingly the ~~right~~ left hand side must also be zero. This may come about in two ways, both of which yield valuable information.

1. The solution $U_1 = u_1$ satisfies this requirement.

From equation (3) $U_1 = \frac{u_2 K_1}{K_2}$,

$$\therefore \quad u_1 = \frac{u_2 K_1}{K_2}$$

$$\text{or} \quad \frac{u_1}{u_2} = \frac{K_1}{K_2} \qquad (6)$$

That is, the mobility ratio of the ion in question is equal to the conductivity ratio, which is determined by all ions. It is further assumed, as a first approximation that the mobility ratio is the same for all ion species, i.e.

$$\frac{(u_j)_1}{(uj)_2} = \frac{(u_j+1)_1}{(uj+2)_2} = \frac{(uj+n)_1}{(uj+n)_2} \qquad (7)$$

Applying equation (5) to all ions other than the one whose mobility is in question, then u_1 for these ions is not equal to U_1; however, from equation (6) the quantity $\left(\frac{K_1}{K_2} - \frac{u_1}{u_2}\right)$ must be zero and accordingly the right hand side of equation (5) must cancel for all these ions. This is only possible if the quantity $(c_1 - c_2)$ is zero (since $u_1 \neq U_1$), so that all ions with mobility different from that of the boundary have equal concentrations on each side of the boundary.

This conclusion does not apply to the ion whose mobility is in question, for which $u_1 = U_1$ and so a concentration change of this ion at the boundary may occur. However, a concentration change of one ion may not occur alone, and therefore, either there is no boundary at all, or there are two ions with the same mobility whose concentration increments (with the signs of their charges) must cancel. The implications of this latter requirement will be considered later.

The alternative solution of the requirement that the quantity $\frac{c_1(u_1 - U_1)}{K_1}$ of equation (4) be zero will now be considered.

2. This solution is clearly

$$c_1 = 0$$

or, if the mobilities on the other side of the boundary are

chosen, i.e. if $u_1 = U_1$, then the condition becomes

$$c_2 = o.$$

That is the ion under investigation must be absent on one side of the boundary, and the conductivity to be used in calculating the mobility is that prevailing on the other side, in the solution containing the ion in question.

This condition is "of paramount importance in determining correct mobilities" (Svensson (7)), and finally settles the question, often raised earlier, as to whether the conductivity of the colloid or of the supernatant solution should be used in calculating mobilities.

(c) The Conductivity Change at a Moving Boundary.

Considering the case of a typical electrophoresis experiment, in which the colloid solution by virtue of its greater density, occupies the lower part of the U tube, it is possible to derive the conductivity changes which are brought about on passing a current.

In this case, the subscript 1 denotes quantities in the buffer, above the boundary, and 2 the corresponding quantities in the colloid solution. U_2 then refers to the mobility of the boundary computed by the conductivity K_2, and U_1 is not required, as it is only the colloid ion which determines the mobility of the boundary; also, for the same reason, the concentration of the colloid ion can be denoted by the symbol C, and its mobility by U ($U = U_2$).

Then equation (5), written in the form

$$c_1 - c_2 = \left(\frac{K_2}{K_1} - \frac{u_2}{u_1}\right) c_1 u_1 \Big/ (u_2 - U_2) \qquad (8)$$

gives the concentration increments at the boundary.

For the colloid ion, $c_1 = o$, $c_2 = C$, $U_2 = U$. Summing for the colloid and for all other ion species (c_j), (c_{j+1}), etc., of mobility (u_j), (u_{j+1}), etc. we obtain

$$-C = \left(\frac{K_2}{K_1} - \frac{u_2}{u_1}\right) \cdot \sum \left((c_j)_1 (u_j)_1 \Big/ (u_j)_2 - U \right) \qquad (9)$$

$$\text{i.e.} \left(\frac{K_2}{K_1} - \frac{u_2}{u_1}\right) \cdot \sum \frac{(c_j\, u_j)1}{(u_j)_2 - U} + C = 0 \qquad (10)$$

The summation does not include $\left(\frac{u_2}{u_1}\right)$, as this is assumed constant for all ion species (equation (7)).

Thus if the leading ion of concentration C were present above the boundary, it would have a mobility U' given by

$$U' = U \frac{u_1}{u_2} \qquad (11)$$

Since it is absent above the boundary, it is immaterial if equation (7) is completely obeyed or not; however equation (11) may be used to simplify equation (10). Considering the quantity $((u_j)_2 - U)$:

Since u_2 for any ion is given by

$$u_2 = \frac{Uu_1}{U'} \qquad \text{(equations (7) and (11)),}$$

$$(u_j)_2 - U = \frac{Uu_1}{U'} - \frac{U'u_2}{u_1}$$

$$= \frac{Uu_1}{U'} - \frac{U'Uu_1}{U'u_1}$$

$$= \frac{U}{U'}\,(u_1 - U'). \qquad (12)$$

Rearranging equation (10) and inserting the value of the mobility difference obtained in equation (12):

$$\frac{K_1}{K_2} - \frac{u_2}{u_1} = C\left(\frac{U}{U'}(u_1 - U')\right) \Big/ \sum (c_j u_j)_1 \quad ,$$

$$\frac{U}{U'} = \frac{u_2}{u_1} \quad ,$$

$$\therefore \left(\frac{K_2}{K_1} - \frac{u_2}{u_1}\right) \equiv -C\frac{u_2}{u_1} \Big/ \frac{\sum (c_j u_j)_1}{((u_j)_1 - U')}$$

and accordingly the final equation for the conductivity change across the boundary becomes

$$\frac{K_2}{K_1} = \frac{u_2}{u_1} \cdot \left[1 - \left(\frac{C}{\sum \frac{(c_j u_j)_1}{(u_j)_1 - U'}}\right)\right] \qquad (13).$$

This equation is of very great importance for the understanding of the boundary migration and the possible anomalies.

It is well known that after electrophoresis the colloid solution possesses a lower conductivity than before (Henry and Brittain (5), Hacker (13), Longsworth and Mc.Innes(14) and by means of equation (13) it is possible to forecast those conditions under which the conductivity difference, and with it the boundary anomalies, may be depressed. The controlling factors are:

(a) The mobility ratio, $\frac{u_2}{u_1}$. This quantity, in most electrophoresis experiments with dilute colloids, is very near to unity, and will not be further discussed.

(b) The concentration of colloid, C. It is evident that high values of C will lead to an increased conductivity ratio and hence greater anomalies. C is expressed in electrochemical equivalents per unit volume, and is therefore $\frac{ZC_{wt}}{M}$ where Z is the valency of the colloid ion, M the molecular weight and C_{wt} the weight concentration. Since the charge to weight ratio is much lower for colloid, than inorganic or simple organic, ions, colloids are much better suited for electrophoresis experiments without pronounced boundary anomalies. In general, experiments should be conducted at the minimum concentration which gives a suitable refractive index gradient.

(c) The magnitude of the quantity $\Sigma\frac{(c_j u_j)_1}{(u_j)_1} = U'$, which shows how the concentration and mobility of the buffer salt

ions affects the boundary anomalies.

In all cases, independent of the sign of the charge on the colloid, the value of the function should be as high as possible. The first requirement is that buffer ion concentration should be high, a well-recognised fact (e.g. Hardy (15)). Furthermore, buffer ions of the same charge as C and U' should have mobilities $(u_j)_1$ as near to U' as possible, giving large values to the function, and counter ions (of the opposite charge) should have low mobilities if these are arithmetically greater than U', giving a value of the function of the same sign as U'; or if arithmetically less, the mobility should be as great as possible, giving a large value of the opposite sign.

In practice, the mobility of the colloid ion is less than those of the buffer ions, and the function has the same sign as U' and C, with the result that the conductivity is constantly lower inside the colloid solution, a fact that has long been known.

The fact that boundary anomalies were less pronounced in buffers of low mobility (especially of the anion) was experimentally discovered by Longsworth, Shedlovsky and McInnes (16) who introduced the use of lithium chloride and diethyl barbiturate solutions.

Section (iii)

The Effects of Conductivity Changes at the Boundary;

The visible boundary anomalies.

(i) The Sharpening and Blurring Effect.

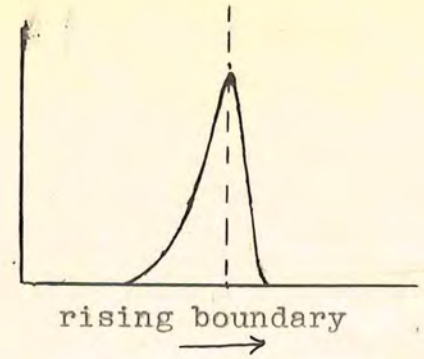
In addition to the facts deduced above concerning the lower conductivity of the colloid solution, this may be further decreased to a slight extent by the Donnan equilibrium, if as is often the case, the colloid solution has been prepared by dialysis against the buffer, which is used as supernatant. The difference in buffer concentration brought about in this way also increases with increasing colloid concentration, and accordingly in the more concentrated solutions the effects of mobility variation caused by gradients of field strength at the boundaries leads to visible deviations from ideal behaviour.

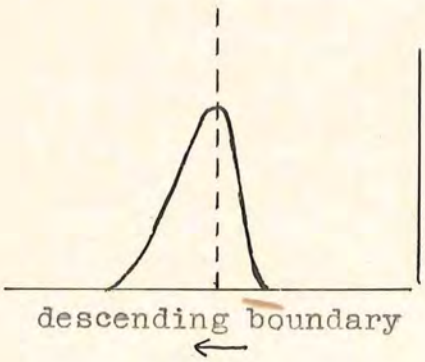
These deviations have been described by Longsworth and McInnes (14), and qualitatively interpreted by these authors thus: The departure, caused by diffusion, of a boundary from its initial sharpness, will be accelerated in the case of a boundary moving (downwards in practice) into a region of increased field strength (i.e. decreased buffer concentration) by the fact that the dilute colloid ions in the uppermost layers will be subject to lower field strengths than those in the concentrated layers in the middle of the boundary, and will therefore lag behind, causing, in this

case, a spreading of the boundary.

The spreading is necessarily accompanied by an artificial sharpening of the other boundary (in general the rising one), but this may be partly or wholly masked if the colloid diffuses rapidly. In this case the colloid ions in the diffuse uppermost layers are in more concentrated buffer, and accordingly are subject to lower field strengths than those in the main body of the boundary, and consequently are overtaken by the latter more rapidly moving ions.

It is evident that this reasoning also explains the loss of symmetry of the concentration gradients in the boundaries. (The optical system, see p.131,of the modern electrophoresis apparatus gives a precise diagram of the variation of the concentration gradients in the limb of the U tube). Considering a boundary rising into a region of decreased field strength, the lower half of the boundary will be in a region of constant field strength, and thus will preserve its original concentration distribution (subject to the diffusion) but the other, upper, half, in a region where there is a field strength gradient of negative sign will be "compressed" or artificially sharpened. A corresponding opposite change occurs in the shape of the other boundary, which is more spread out in the direction in which it is moving. The resulting patterns are exemplified in the diagram.





Also, as is evident from the diagram, the ordinate of maximum concentration gradient does not divide the area under the curve into halves. For mobility determinations, as Longsworth and McInnes have pointed out, it is better to take the boundary as being located at the position of the ordinate which bisects the area, as this represents the position which the boundary would occupy had it retained its original sharpness.

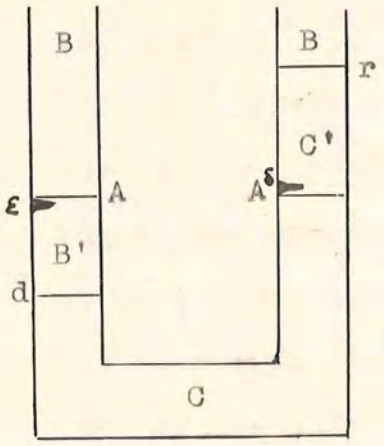
(ii) <u>The "δ" and "ε" boundaries; The greater mobility of the advancing boundary.</u>

The stationary δ boundary, first discovered by Tiselius in 1937 (8a) has been proved to be an example of a further anomaly met with when more concentrated colloid solutions are used. It is now known that the δ boundary is due to a gradient of buffer salt composition, with a super-imposed colloid concentration gradient. The ε boundary, the small stationary boundary left behind at the original position of the descending boundary has a similar nature, without, of course, a colloid concentration gradient.

These anomalies are best considered with the aid of the accompanying diagram, which represents a typical electro-

phoresis experiment.

A-A represents the position of the boundaries at the commencement of electrophoresis, r and d the positions of the rising and descending boundaries respectively, after electrophoresis has proceeded for some time.



At the descending boundary the colloid concentration changes to zero, and consequently in the boundary also there is an equal and opposite (with regard to electrochemical concentrations and signs) change in concentration of the buffer, an effect which has been considered in section (ii) of this chapter.

That is, the intervening volume of buffer solution, between the boundary d and its original position, acquires an adjusted composition B', determined by the value of its "regulating function", which Kohlrausch defined as the sum of all ionic concentrations divided by the respective sum of all their mobilities. This solution (B'), in which the concentration of buffer ions is slightly greater than in the colloid solution, is thus a region of lower field strength than that prevailing in the colloid, and accordingly the descending boundary, migrating into a region of increased field strength, is spread out with the progress of electrophoresis, in agreement with experimental practice.

The greater density of the colloid solution (of slightly lower buffer concentration than the solution above it) keeps the system stable.

Since, however, the concentration of buffer in the main bulk of both supernatant and colloid solution is constant (or differs only slightly, if Donnan equilibrium has been attained) there is a change in concentration of buffer at the original position of the descending boundary, corresponding to the one occurring in the boundary d, i.e. located at ε , there is a concentration gradient, where the buffer concentration (B') of the intervening solution reverts to that of the buffer (B). Thus a stationary (or very slowly-moving) boundary appears at ε , where two slightly different concentrations of buffer meet. This boundary is also gravitationally stable.

Longsworth and McInnes have measured the conductivities of the various parts of the solution after electrophoresis, and find in agreement with the above, that $K_{B'} > K_B > K_C$.

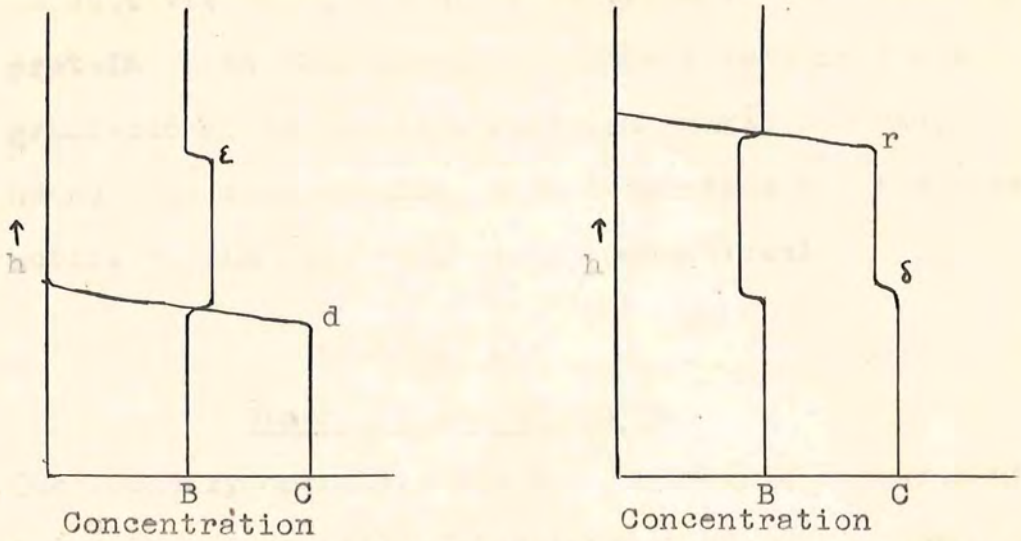
A somewhat similar change occurs at δ , the original position of the rising boundary. Owing to the change in buffer concentration at the boundary r (where the colloid concentration drops to zero) there is a slight decrease in concentration of buffer ions in the intervening volume of solution of composition C'. At δ , the buffer concentration has a corresponding increment, and regains its constant

value. Since, however, the intervening solution (C') has a lower buffer concentration than that below δ, the field strength is correspondingly increased, and the velocity of the colloid particles in the solution C' will be greater than the velocity of those in the main bulk of solution (C), i.e. the ascending boundary has an increased velocity, and an apparently increased mobility, if the conductivity changes below the boundary are not taken into account. This instance of anomalous migration was noticed by Henry and Brittain (5) working with silver sols.

Thus at δ, there is a gradient of buffer salt concentration causing a stationary (or very slowly-moving) boundary. The concentration is greater below the boundary than above it, rendering the system gravitationally stable. Since the concentration gradient involves a field strength gradient, the velocity of the colloid particles is increased above the boundary, and consequently their concentration is decreased, so that there is in addition a gradient of colloid concentration superimposed on the salt gradient at the boundary. This accounts for its greater visibility compared with the ε boundary.

Since the conductivity of the solution C' is less than that of B, the rising boundary is migrating into a region of decreased field strength, and accordingly is sharpened. The concentration changes in buffer salt and colloid have

been graphically represented by Longsworth and McInnes, from whose work the accompanying diagrams have been derived.



B = Concentration of buffer, C = concentration of colloid, h = height in the U tube limb.

In addition to verifying experimentally the concentration changes, these authors have also derived the necessary corrections to the measured velocity and area of the rising boundary which must be applied before the true mobility of the leading ion can be determined. When this has been done, the mobility of the ascending boundary is identical with that of the descending boundary.

In final proof of the nature of the δ and ε boundaries, which were at first thought to be due to slowly-migrating protein or carbohydrate constituents, the above investigators performed an experiment in which the dialysed protein solution was diluted with water before electrophoresis, the

dilution being such as was indicated by the final conductivities of a similar experiment with undiluted protein. In this case, where there were no field gradients at the initial position, neither δ nor ε boundaries were present, both boundaries had the same mobility, and were very nearly symmetrical.

Section (iv)

Other Sources of Error.

The boundary anomalies are the most serious source of error in the accurate determination of mobilities, and accordingly have been treated in some detail, so that the conditions for their elimination or reduction to insignificance may be well defined. However, there are other possible sources of error, which, with their means of depression, will now be considered.

(a) The Electrode Reactions.

In practice reversible silver-silver chloride electrodes, surrounded by saturated potassium chloride solution, are always used, and consequently there is no chance of acid or alkali being formed. The only consideration is that of the effects of migration of the potassium and chloride ions. This has been examined by Tiselius (8).

The total amount of separation of two components A and B (i.e. the volume of pure A or pure B) of a colloid mixture,

of mobilities u_A and u_B, caused by passing a current i for a time t is independent of the dimensions of the apparatus, and is given by $(u_A-u_B)Xtq$ where X is the field strength and q is the cross sectional area of the limb of the U tube. This quantity is the volume of pure A or pure B, and since $X = \frac{i}{qK}$ where K is the specific conductivity, is independent of the apparatus constants.

At the same time, the quantity of electricity it causes a movement of the electrode solution ions of mobility u through a volume V, given by

$$V = \frac{u\,i\,t}{K'}$$ where K' is the conductivity prevailing in the electrode solution (in general, $K' \gg K$)

It is therefore evident that in order to prevent the ions of the electrode solution reaching the moving boundary, there must be a volume V' of intervening buffer solution at least equal to $\frac{u\,i\,t}{K'}$.

The quantity of electricity it, sufficient to cause a complete separation of the two components A and B, can only be passed, therefore, if

$$V = \frac{u\,i\,t}{K}$$, assuming K'=K, the least favorable case.

But V_A = volume of pure A(or B) = $\frac{(u_A-u_B)it}{K}$

$$\therefore \quad V' = \frac{u\, V_A}{u_A - u_B}$$

This quantity is the minimum value of V', and in practice it is made twice as great. In the present series, it was found that V' was ten times as large as necessary, as only one component, of relatively high mobility, was encountered.

NB. Also possibly ∫ charge ∫ volume of different electrode - depending on partial specific vol. ∫ Ag - Cl ... v AgCl

(b) The Free Liquid Surface.

Owing to the density difference between the colloid and buffer solutions, a shift of the colloid solution from its original symmetrical position in the bottom of the U tube produces a hydrostatic pressure

$$\left(\rho_{(colloid)} - \rho_{(buffer)} \right) hg,$$

where h is the difference in height of the boundaries. Correspondingly, for equilibrium, the level of liquid in one electrode vessel is greater than that in the other by an amount H given by

$$\rho_{(buffer)} \cdot H = h \left(\rho_{(colloid)} - \rho_{(buffer)} \right)$$

$$\therefore \quad H = h \cdot \frac{\rho' - \rho}{\rho}$$ where ρ' is the density of the colloid.

This height difference at the electrode vessels corresponds to a decrement in h, at the boundaries, in the ratio of their respective cross sectional areas, S and q.

That is $$\Delta h = \frac{S}{q} \cdot h\left(\frac{\rho' - \rho}{\rho}\right) .$$

Consequently the measured distance $\frac{h}{2}$ moved by one boundary should be increased by half the quantity Δh. It is evident that in order to keep this correction as small as possible, S should be small, and dilute solutions of colloid used. In the present investigation, where 0.20% solutions of sodium deoxyribonucleate were used generally, the correction was always less than $\frac{1}{2}\%$.

(c) The Electro-osmosis.

The motion of the whole liquid relative to the walls, caused by their difference in charge is, as Tiselius (4) has indicated, negligible in tubes as wide as the ones used in the apparatus.

(d) The Heating Effect of the Current; Convections.

It is desirable, as has been shown earlier, to use high concentrations of electrolyte in order to minimise the boundary anomalies; further it is often desirable to pass comparatively high currents so that the boundaries move comparatively rapidly, and electrophoretic separation is brought about before the boundaries become too diffuse.

Both these factors, high electrolyte concentration, and high current strengths, involve large heating effects which disturb and may destroy the boundaries by convection; consequently a mean must be struck whereby the migration is sufficiently rapid for the desired separation to occur,

while the heat developed is not so great as to cause disturbances. These effects were investigated by Tiselius (4,8), and the great precision with which electrophoretic analysis can now be effected is largely due to his suggestion of working at or near the temperature of maximum density of water, and the use of flattened rectangular sections of the U tube, whereby a better heat exchange is possible between the solution and the thermostat water. In practice, the thermostat regulates at 0.5°C, and consequently a temperature rise of 2 or 3° may occur before the density begins to decrease and convections occur, and it is now possible to use adequate power in the apparatus.

If an excessive power is applied and convections do occur, the boundaries, instead of being horizontal, become convex or concave, and appear on the screen artificially sharpened or blurred. In extreme cases, an apparent separation of a boundary may be observed, owing to the presence, at two slightly different levels in the U tube limb, of refractive index gradients. In general, the power used in the present investigation was 70-80% of that at which convections set in.

The only other source of convections is that which may occur if any boundary is allowed to pass into the bottom joining-section of the U tube, when its density gradient becomes unstable. Such a motion must consequently be avoided.

Section (v)

(a) The Tiselius Electrophoresis Apparatus.

The apparatus used in the present investigation was a modification of that described by Tiselius (8) and was supplied by Messrs. Adam Hilger Ltd. Approximately scale diagrams of the U tube and of the whole assembly in its carriage are shown in figures 2A and 1 on p.123 respectively.

The U tube, in which the boundaries are formed and observed consists of the four sections I - IV, which may be slid over each other along the planes a - a', b - b', c - c'. The surfaces of the sections of the shape shown in fig. 2B are ground planar, and are greased before assembly, which is performed in the following order.

The bottom joining piece of the U tube, IV, is placed on its fitted rubber pad, and secured in position at the bottom of the carriage. The next piece III is then slid over it, and worked to and fro until the air bubbles between the greased surfaces have been expelled. Then this part of the U tube is filled with the (cooled) colloid solution so that the menisci appear on the surface of the piece III, above the level of the top. The next piece II is then slid on in such a manner that the superfluous liquid of the menisci is pushed away; the piece II is kept displaced out of alignment so that the sections III and IV are completely full and sealed off. The section III is

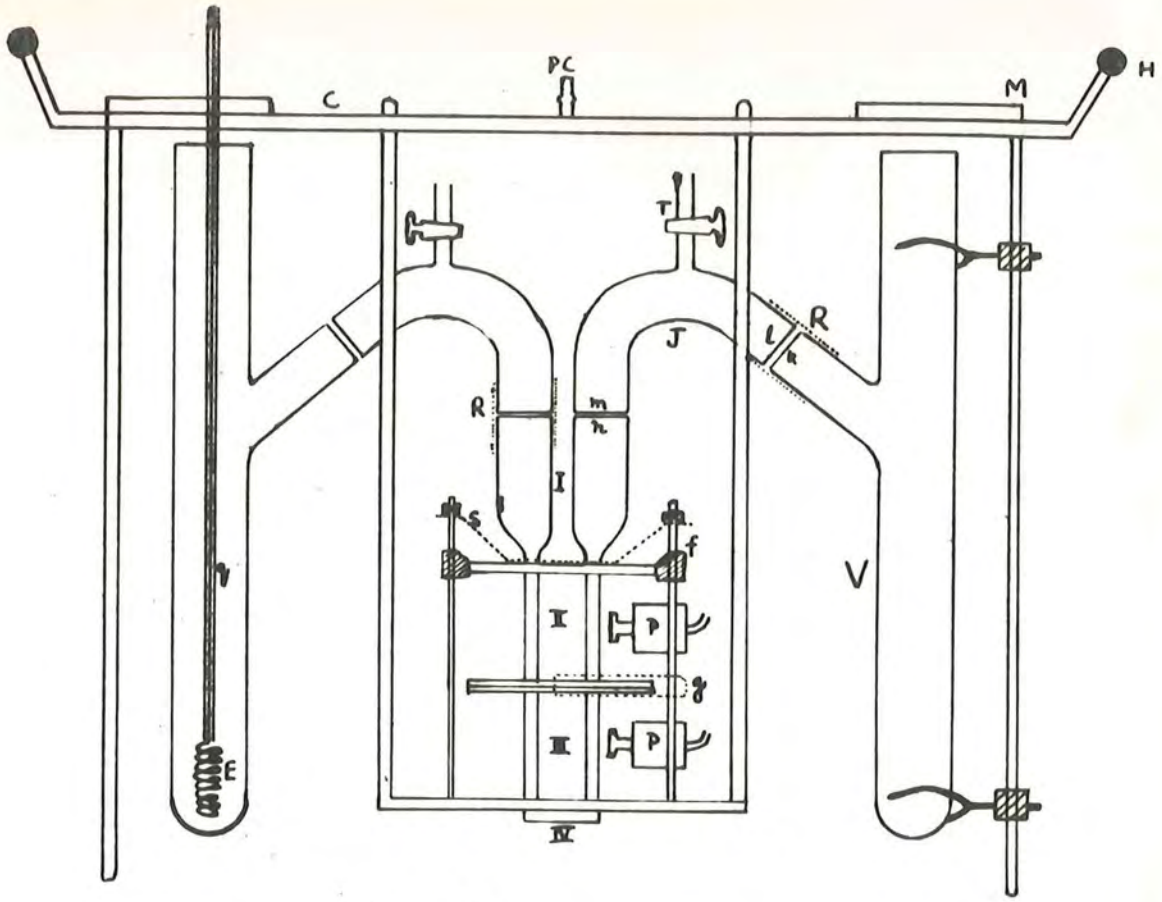


Figure 1.

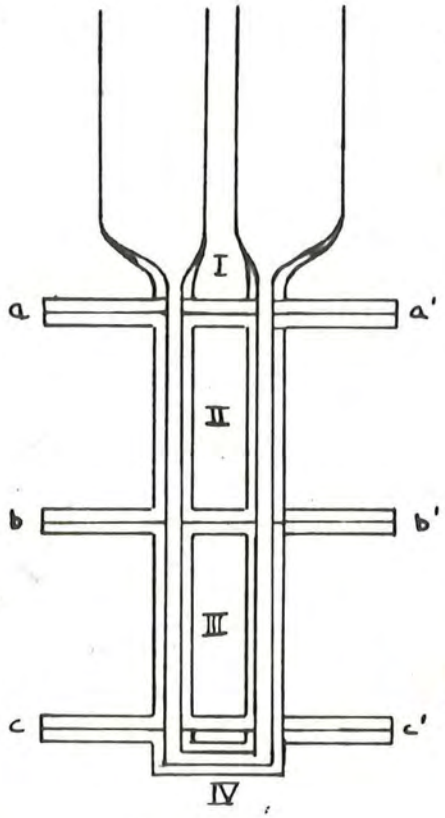


Figure 2A

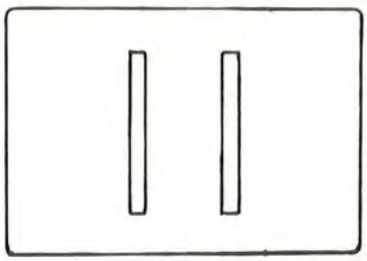


Figure 2B

then displaced together with Section II, so that the latter is brought into alignment with section IV, while section III is completely sealed off. Then the top section I is slid over, in alignment with sections II and IV. This method of filling the U tube is more rapid, and with surface active materials and viscous solutions it is more efficient than the alternative method, whereby sections II, III and IV are assembled and filled with colloid to a level above b - b', section III is moved out of alignment and sealed off, and the excess colloid solution removed from the section II by rinsing with buffer.

The U tube is then clamped in position: the small block f (fig.1) holds section I rigid, the spring clips g are pressed against the common edges of sections II and III (b - b' in Fig. 2A) with a slight pressure inwards (so that these sections are kept square when section III is moved into alignment), and finally the spring clips S are fitted, which bear on the upper surface of section I, keeping the whole U tube assembly firm.

The next step is the fitting of the electrode assembly. The electrode vessels (approximate capacity 500 ml. each), which have ground surfaces at k are first joined on to the joining-pieces J (which also have ground surfaces at l and m) by means of close fitting rubber tubes R, and another rubber tube is rolled on to the other end of the joining-

piece above m. The electrode vessel is then placed in its mounting M, and the end m of the joining-piece brought into juxta-position with the ground surface n of the section I of the U tube. The rubber joining tube is then rolled down, securing m and n in intimate contact, and the assembly of half the apparatus is complete. The other electrode vessel is similarly fitted and the apparatus can then be filled with the buffer. This is done by pouring the buffer solution with each electrode vessel, with the taps T of the side arms of the joining-pieces J open, so that the buffer fills the apparatus from the bottom of section II of the U tube.

The whole assembly is then placed inside the thermostat, the carriage C (fitted with handles H) locating on runners at the top of the thermostat. The electrodes E, which consist of a double coil of silver wire coated with silver chloride, with an outer ebonite insulating sheath q are then placed in the electrode vessels; the apparatus is then left for at least two hours to attain equilibrium.

The thermostat consists of a (black painted) aluminium tank of some 80 litres capacity, surrounded by a double-walled wooden case, the space between being well lagged with kapok. The intervening spaces between the windows of the tank and those of the case are kept dry by dishes

of phosphorus pentoxide, which prevents condensation on the inner surfaces of the windows: the outer surfaces are kept dry by a current of hot air from small heaters mounted below the windows of the case.

The thermostat is kept well stirred by two large "propeller-shaped" stirrers, these being operated by a pulley system from a motor mounted on the stone bench on which the thermostat rests; no vibration was detectable.

After two hours, ice-cold saturated potassium chloride solution is run into each electrode vessel (about 80 ml. each), by a tap-funnel reaching to the bottom of the vessel. (The excess buffer displaced is removed with a pipette). A T-piece is then joined on to the side arms of the electrode vessels, the taps opened, suction applied until junction of the solutions from the vessels is achieved, and the heights of liquid in the electrode vessels allowed to become level. The taps T are then closed, and the T-piece removed.

The section III of the U tube is then moved back into alignment, so that a continuous passage through the apparatus is formed, and the boundary between colloid and buffer solutions made. The movement of the section is brought about by applying gentle pressure to the pneumatic pump, P, which is connected by a rubber tube to the pump connection PC at the top of the carriage. This arrange-

ment, originally devised by Tiselius, is well suited to the purpose: the gentle pressure which reaches the walls of the U tube sections can never cause cracks.

The boundaries are then displaced into view from behind the horizontal faces of the U tube sections, either by "compensator action" (whereby a metal cylinder is lowered extremely slowly, by a geared down synchronous clock motor, into the electrode vessel), or, as in the present investigation, by the extremely slow addition of a small amount of buffer solution to one electrode vessel, this being accomplished with the aid of a pipette drawn into a very fine capillary. The latter method was very much more convenient, and was never found to disturb the boundaries.

The apparatus is now ready for electrophoresis to be commenced; the electrode leads are clipped on to the electrodes, a suitable current passed from the rectifier (two ammeters were fitted into the electrode circuit, one on either side of the cell), and photographs of the positions of the boundaries taken at suitable intervals of time.

The above description concerns the assembly of the medium form of the apparatus, which uses about 10 ml. of colloid solution and is the most convenient for mobility determinations. It applies in principle also to the use of the large scale form of the instrument, which is used for the electrophoretic separation and isolation of the components of colloidal mixtures.

In this case, however, the U tube sections are very much larger, and there are four middle sections instead of the two of the medium form (i.e. sections II and III) and about 150 ml. of colloid is required. As the electrode vessels are too large to fit inside the thermostat (they are of 6 litre capacity each) they are mounted outside, and connection made by glass tubes and rubber joiners to the top of the U tube. For the separation of, for example, two components of mobility u_A and u_B ($u_A > u_B$) the current is started and then the whole solution is slowly displaced by compensator action with a velocity equal and opposite to the arithmetic mean of u_A and u_B. By this means at the end of the run pure A can be isolated from the sections above the original boundary on the one (ascending) side, and pure B from the sections above the original boundary on the other side.

(b) Observation of the Boundaries.

It is in the method of observation of the boundaries that the most striking improvements have been made since Tiselius' original apparatus: the optical system of the apparatus in use was of the type originally devised by Philpot (10) whereby a cylindrical lens is used in conjunction with a diagonal straight edge to give on the screen a complete plot of the refractive index gradients in the boundaries as a contrast between dark peaks and light background.

Only the principles of the method will be considered, as for the purpose of mobility determinations with homogeneous colloids it is only the accurate location of the boundaries that is of importance. For electrophoretic analysis, whereby all the components of a relatively complex mixture may have their concentrations and mobilities determined, a complete treatment of the relationship of the electrophoretic pattern, the positions of the adjustable parts of the optical system, and the actual concentration gradients existing in the boundaries, would have to be considered. Such treatments have been furnished by Longsworth, (Optical Methods in Electrophoresis (17)) and by Svensson (7).

The Foucault-Toepler Schlieren Method.

Fig.3. p.131, after Longsworth (17) illustrates the principles of the Foucault-Teopler 'lens-imperfection' test.

Light from a point source S illuminates the lens L, which has a slight imperfection, idealised as a minute prism, at A. The light passing through all other points of the lens forms an image of S at the point P, but those rays passing through A are deviated further, and this portion of the lens produces another image of S at Q. The objective O of a camera is placed just to the right of P and focussed on L, thereby forming an image of the lens on the screen at G.

Since it is a property of the camera objective that all rays originating in its object plane are brought to a focus at conjugate points in its image plane, regardless of the portion of the objective through which they pass, the imperfection A is not visible on the screen at G. If, however, the opaque screen D is raised so as to intercept the light forming the image at Q, while allowing that forming image P to pass, then the imperfection will become visible at A' as a dark shadow, or "schlieren" in an otherwise uniformly illuminated background.

The application of this method to the observation of the refractive index gradients encountered in electrophoresis is shown in Fig. 4, p.131.

The point source of light is replaced by a thin horizontal slit S, and the U tube containing the boundary is placed close to the schlieren lens L : the shading in the U tube represents the regions (at the boundary) where the refractive index is varying with the height. Gravity ensures that the density, and hence the refractive index are uniform in each horizontal layer in the boundary. The schlieren diaphragm D is replaced by a diagonal slit (or straight edge) I, whose angle may be adjusted. In addition a cylindrical lens is placed at C, and is focussed on the diagonal slit and the camera screen G. The camera objective O is focussed on the

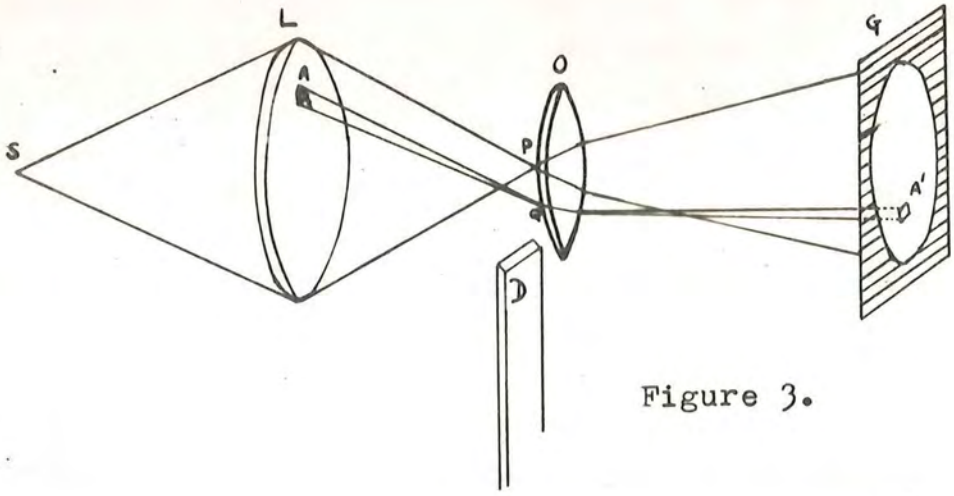


Figure 3.

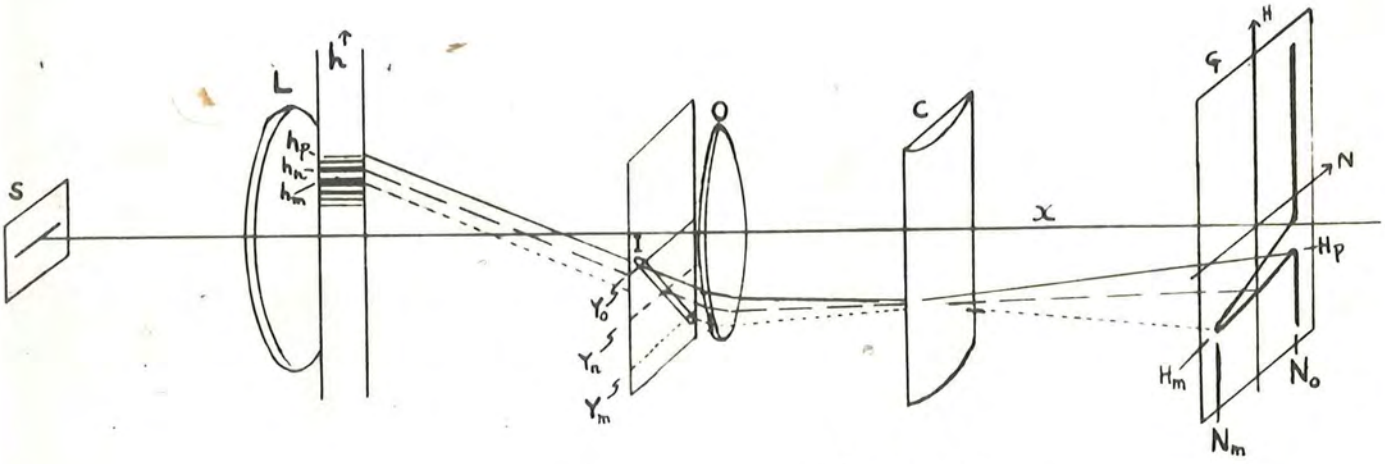


Figure 4.

Figure 5

U tube, and also forms an image at G - it is evident that the vertical coordinate of a ray passing through the cylindrical lens C is unaltered, but the ray may be deviated laterally along the cross axis N.

If there is no boundary in the limb of the U tube, all the light passing through it is concentrated in the normal slit image at Y_o. As is evident from fig. 4., only the light forming the extreme left hand part of this image can pass through the diagonal slit. In the figure these rays are to the left of the optic axis x and are therefore deflected to the right of the axis by the cylindrical lens, forming an image on the screen at N_o. Other rays, in the absence of a boundary, undergo similar deviations, with the result that a narrow vertical band of light is formed on the screen upwards from N_o. This is the base line of the pattern.

The deviations occurring when a boundary is present in the tube are shown by tracing the paths of three rays. One, passing through the homogeneous solution h_p, behaves as before, passing through Y_o and forming one element of the base line at N_o. The pencil of light passing through the layer h_m of maximum refractive index gradient is deflected more than the first ray, and forms an image of the slit at Y_m. In this case, however, only the extreme right hand portion of the light can pass through the diagonal slit, and

is thus deflected to the left of the optic axis by the cylindrical lens, forming the peak of the pattern at N_m. The camera objective would bring this pencil to focus at the same level in the absence of the cylindrical lens, whose function, therefore, is to deviate laterally those rays which have been vertically deviated by the refractive index gradient at the boundary. The path of a third ray, passing through a part of the boundary other than that of the maximum refractive index gradient, is indicated by the dashed line. The image of the slit is then formed at Y_n, intermediate between Yo and Y_m ; consequently, the middle portion only of this image is passed by the slit, no deviation is suffered at the cylindrical lens, and the camera objective brings it to focus on the centre of the screen, intermediate between N_o and N_m. All other rays passing through the boundary undergo similar deflections, being deviated by the cylindrical lens to an extent proportional to their deflection by the refractive index gradient at the boundary, and hence the complete electrophoretic pattern is built up on the screen. Thus this pattern, which appears in the shape shown in Fig. 5A, p.131, is a plot of the refractive index gradient in the tube against the corresponding height in the tube; for purposes of publication, it is usually transposed to the vertical position, shown in Fig. 5B.

It is evident that if a diagonal straight edge is used

instead of a slit, and the edge is brought up from the bottom left of diagram 4, then all the light forming the image Y_o will be allowed to pass, forming a bright horizontal line on the screen from N_o to N_o' ; similarly, of the light forming the intermediate image Y_n, only the right hand half can pass, forming a bright line on the left hand half of the screen, i.e. the image on the screen consists of the same plot of refractive index gradient against height, but this now is visible as a dark peak against a light background, as shown in Fig. 5C.

This latter arrangement was generally used in the present investigation, and gave extremely sharp boundary definition at angles of the edge of about 50^o from the vertical.

It is evident that if the diagonal straight edge had been brought down from the right hand side of the diagram, the image of the pattern would have been bright against a dark background. This arrangement was not so sensitive for accurate location of the boundaries.

The optical arrangement shown in Fig.4 was modified slightly in practice. The light source S consisted of a mercury vapour lamp with a green filter, and the width of the slit was adjustable (wide openings gave very bright illumination, with, however, some loss of definition). The schlieren lens L was placed at a distance from the ~~water bath containing the U tube~~ light source equal to its focal length; the light leaving the lens was thus

parallel. A second schlieren lens on the further side of the water bath was so placed as to focus the parallel light on the schlieren diaphragm. Two mirrors were introduced on the side of the thermostat away from the light source, both at 45°, so that the light was returned in a direction parallel to the original. By this means the camera was placed by the side of the water bath, and the whole apparatus, which was supported on a concrete bench built into the main structure of the building, made much more compact.

Section (vi)

Summary

It is now convenient to summarise those conditions under which mobilities may be determined in the absence of pronounced boundary anomalies.

(a) The ion in question must be absent on one side of the boundary. This is more important for crystalloid than colloid ions, and in any case is general electrophoretic practice.

(b) High buffer salt, and low colloid, concentrations must be used. This was not inconvenient in the present investigation, as the specific refractive index increment of sodium deoxyribonucleate is relatively high, and its mobility is also fairly great. The use of buffers whose ions have high mobilities should be avoided.

(c) If visible δ and ϵ boundaries are present, the conductivity of the colloid solution after electrophoresis must be used for mobility determinations on the rising boundary; it is generally preferable in such cases, to determine the mobility from the more diffuse descending boundary.

In the present investigation, under the conditions stated in (b) above, no appreciable boundary anomalies were detectable, and the conductivity was sensibly constant throughout the tube. Under these conditions, the velocity of both boundaries is constant, and is given by

$$V = F.U$$

The field strength F is given by $F = \frac{E}{d}$, where E is the applied potential, and d the distance between the electrodes.

Also, $E = Ri = \frac{di}{qK}$, where R is the total resistance, i the current, q the cross-sectional area of the U tube, and K the specific conductivity. That is $F = \frac{i}{qK}$,

$$\therefore \quad U = \frac{VqK}{i} = \frac{x\ q\ K}{i\ t} \quad ,$$

where x is the distance travelled by the boundary in the time t. This latter equation was used for the computation of mobilities.

REFERENCES TO CHAPTER V.

1. Picton and Linder J.C.S. 1897, 71, 568.
2. Svedberg and Andersson Koll.Z. 1919, 24, 156.
3. Kruyt. Koll.Z. 1925, 37, 358.
4. Tiselius. Nova Acta Regiae Soc.Sci.Upsaliensis IV, 1930, 7, No.4.
5. Henry and Brittain, Trans.Farad.Soc. 1933, 29, 798.
6. Dole, J.A.C.S. 1945, 67, 1119.
7. Svensson, Arkiv.Kemi.Min o. Geol. 1946, 22A, No.10.
8. Tiselius, Trans. Farad. Soc. 1937, 33, 524.

8(a) " Biochem. J. 1937, 31, 1464.

9. Longsworth, Chem.Rev. 1942, 30, 323.
10. Philpot, Nature 1938, 141, 283.
11. Kohlrausch, Ann.Physik.Chem., 1897, 62, 209.
12. Weber, Sitzungsberichte. Akad.Wissensch.Berlin 1897, 936.
13. Hacker Koll.Z. 1933, 62, 37.
14. Longsworth and McInnes, Chem.Rev.1939, 24, 271; J.A.C.S. 1940, 62, 705.
15. Hardy, J.Physiol. 1905, 33, 251.
16. Longsworth, Shedlovsky and McInnes. J.Exp.Med. 1939, 70, 399.
17. Longsworth, Ind. and Eng.Chem. 1946, 18, 219.

Chapter VI

The Electrophoretic Properties of Sodium Deoxyribonucleate.

Section (i) General.

(ii)Mobility-Concentration Relationship.

(iii)Mobility-Ionic Strength Relationship.

(iv)Mobility-pH Relationship.

Section (i)

General Electrophoretic Properties; Results obtained in Previous investigations.

In common with other preparations of sodium deoxyribonucleate whose electrophoretic properties have been examined (L-5), for review see (7), the material used in the present investigation was electrophoretically homogeneous, no separation of the main migrating boundaries being detectable under the widely varying conditions of pH and ionic strength employed.

In conformity with the high molecular weight of the substance (Chapter VII) diffusion occurred slowly, the boundaries remaining very sharp throughout the runs, which extended for periods of 1½-4 hours. In general, a colloid concentration of 0.20% was maintained, this being sufficient to give very well defined boundaries, but insufficient to cause noticeable anomalies at the (generally high) ionic strengths used. As is evident from the electrophoretic patterns reproduced on Page147, no appreciable δ or ε boundaries are visible, the distances moved by both rising and descending boundaries are sensibly the same, and the descending boundaries are not unduly blurred. Accordingly the system is suitable for the direct measurement of mobilities, which were determined in some experiments by measuring the distances travelled by both ascending and descending boundaries;

it was found, however, that these distances were identical within the limits of experimental error, and accordingly, for most experiments, the distances covered by the rising boundary only were measured, as this boundary was somewhat sharper. The mobility values were not corrected for differences of solvent viscosity, in view of the doubtful validity of this correction (Svensson (8)).

Experiments were carried out to establish the mode of variation of the mobility, (a) with concentration of sodium deoxyribonucleate, (b) with ionic strength at a constant pH and colloid concentration and (c) with pH at a constant ionic strength and colloid concentration. With these data available, a colloidal substance may be considered to be adequately characterised from the electrophoretic viewpoint. Although Stenhagen and Teorell (1) measured the mobility over restricted ranges of pH and colloid concentrations, there is, apparently, no record of the effect of variation of the ionic strength. Thus the various reported values of the mobility at pH 7.0 (Table I) shew rather large discrepancies, which Cohen (2) assumed were due to differences in charge caused by variations in the amount of ionised phosphoryl groups. In view of the implications from the viscosity and electrometric titration data discussed in Chapter IV, that many of the substances previously examined (including those prepared by the Hammarsten-Bang method) had been to some extent degraded, the mobility

data of this sample, which was monodisperse with regard to electrophoresis and sedimentation (See Chapter VII) should prove of especial interest.

Table I

Recorded Values of the Electrophoretic Mobility (at pH 7.0) of Calf Thymus Deoxyribonucleic Acid.

Investigator	Method of Extraction of Material	Ionic Strength	Buffer	Mobility*
Stenhagen and Teorell (1)	Hammarsten-Bang	0.10	Phosphate	1.9
Cohen (2)	Hammarsten-Bang	0.10	Veronal	1.7
	Levene	0.10	Veronal	1.4
Hall (3)	Hammarsten-Bang	0.02	Phosphate	2.1
Seibert (4)	(Deoxyribo-acid from tuberculin protein)	0.02	Phosphate	2.2
Zittle and Seibert (5)	(Deoxyribo-acid from sonically disintegrated streptococci. Material contaminated with ribonucleic acid)	0.10	Phosphate	1.2 - 1.3
Present investigation (6)	Gulland, Jordan and Threfall (Chapter III) (Sample T5/1)	0.20	Phosphate	1.43
	Alkaline (similar to Levene process) (Sample TC/1).	0.20	Phosphate	1.30

*(These, and all subsequent, mobilities are in terms of microns/sec./(volt/cm.) after the suggestion of Abramson et al (9), in order to avoid possible confusion caused by

the use of negative indices.)

Section (ii)

The Variation of the Mobility with Sodium Deoxyribonucleate Concentration

In view of the great increase in viscosity with increased colloid concentration in this instance, the relation between the mobility and the concentration at constant ionic strength is a question of considerable interest; the experiments of Freundlich and Abramson (9) indicated that the mobility of gelatin did not vary during the sol-gel transition, when the viscosity of the solution increases many times, and, therefore, these authors considered that the controlling factor was the viscosity of the dispersion medium alone.

The mobility of thymus nucleohistone, which also gives very viscous solutions, was found by Hall (3) to depend on the concentration, but the mobility values he quotes cannot be of great accuracy, as they were averages of the mobilities of the ascending and descending boundaries, between which considerable discrepancies occurred as the concentration was increased.

The experiments of Stenhagen and Teorell indicated that the mobility of the samples of sodium deoxyribonucleate used by them was independent of the concentration, even though the viscosity underwent a threefold change over the range of concentration investigated.

This result has been verified in the present investigation.

The results obtained are recorded in Table II. It is evident that no significant change in the mobility is caused by increasing the concentration, even though the viscosity varies very greatly.

Table II

Mobility-
Concentration Relation for Sodium Deoxyribonucleate

Concentration	Mobility	Approximate Relative Viscosity at 8000 dynes/sq.cm.
0.05%	1.39	3
0.10%	1.44	7
0.20%	1.43	21
0.30%	1.50	40

Ionic strength = 0.20 in all cases.

It was found impossible to experiment with solutions of greater concentration than 0.30% owing to the very high viscosity.

In contrast to the results obtained by Stenhagen and Teorell, the mobility under all conditions did not vary appreciably with the field strength, values of the quantity $(\frac{\Delta x}{i\Delta t})$ (where Δx is the distance traversed by this boundary in time Δt, and i is the current) being very nearly independent of the particular value of the current.

The approximate constancy of the mobility with increasing solution viscosity is in agreement with expectation from electrokinetic theory

electrokinetic theory (See Chapter VII); at the high value of the ionic strength, the thickness of the double layer is small ($\frac{1}{K}$ = c7A) and its properties will approximate to those of the solvent. The viscosity of the latter is thus the controlling factor, as in the case of the soaps and other gels quoted by Stenhagen and Teorell.

Section (iii)

The Variation of the Mobility with Ionic Strength

The relation between the mobility of a colloidal particle and the ionic strength of the solution is of fundamental importance in electrokinetic theory, and the question is reviewed in the next Chapter.

The results obtained in this investigation are shewn graphically in Figure 6/1. The mobility values at the lower ionic strength, when slight anomalies became visible, were determined on the descending boundaries. They cannot be considered to have the same accuracy as those in the range 0.30 - 0.05.

It is at once evident from the graph that the mobility of the substance is greatly influenced by the ionic strength; this variation also accounts, in part, for the discrepancies in previously recorded values whewn in Table I, at least for the samples extracted by mild methods. It would appear, however, that in agreement with expectation, the mobility values of materials extracted by drastic methods (e.g. Levene procedure) are slightly lower. This phenomenon can probably be related to

the phosphorus deficiency of these materials, as suggested by Cohen.

The experiments recorded in this section, and also in Section (ii) were carried out in unbuffered solutions, the ionic strength being due entirely to sodium chloride. As the substance itself is unbuffered at neutrality, there is no appreciable mobility change across the boundaries, which remained free from anomalies. The quantitative interpretation of mobility values is greatly facilitated by conducting electrophoresis in solutions of symmetrical electrolytes whose hydrated ionic radii are small and well established.

Section (iv)

The Variation of the Mobility with pH

This variation is an extremely important property of a colloidal ampholyte, the pH mobility curve ranking with the base binding curve in the ability to provide information as to the nature of the functional groups of the particles in solution.

The accurate determination of the pH mobility relationship for this sample of sodium deoxyribonucleate was one of the most important aspects of the present investigation: consequently the theory forming the basis of accurate mobility determination with the Tiselius instrument has been reviewed in some detail. (Chapter V).

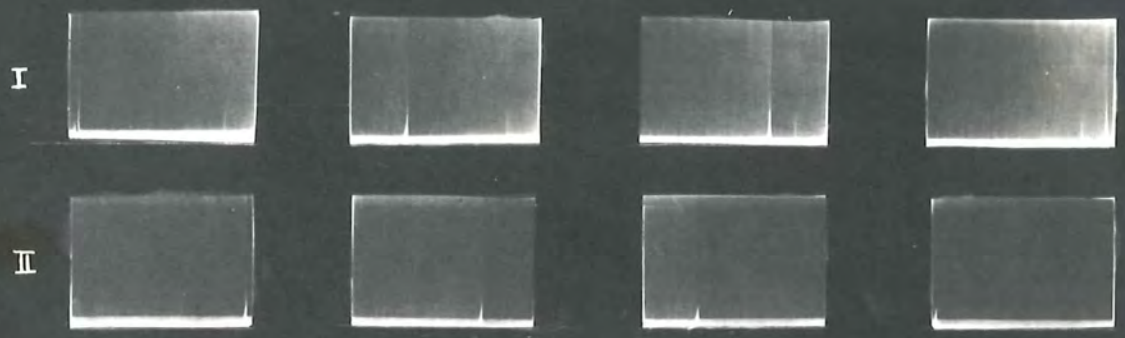
The results obtained are shewn graphically (Graph 6/2), and a representative series of electrophoretic patterns obtained

at various pH's is shown on pl47&8. It is evident that the substance is electrophoretically homogeneous over the complete range studied. The curve was obtained at a constant ionic strength of 0.20, uni-univalent buffers being used wherever possible, and organic buffers (such as glycine) being avoided.

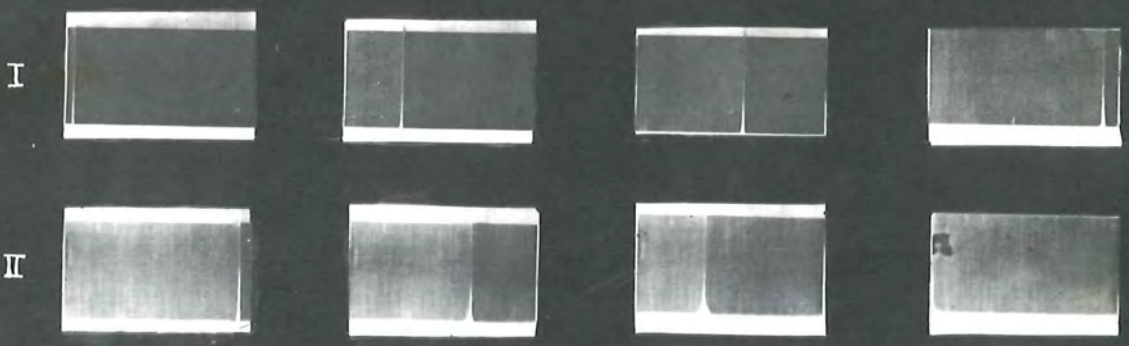
Stenhagen and Teorell (1) published an incomplete form of the pH- mobility, and acid-base binding, curves for their sample of sodium deoxyribonucleate, but as their measurements were restricted to the pH range 3.5 - 10 they did not observe the great increase in mobility at pH's greater than 10. The first part of the curve obtained in the present investigation agrees well with the one recorded by these investigators, if allowance is made for the different ionic strengths employed. Stenhagen and Teorell noted the close resemblance between the pH- mobility, and acid-base binding curves for their material. It is evident from a comparison of the mobility curve (solid line, Graph 6/2) with the back - titration curve (dotted Line) also shown on the graph, that the general similarity is also shown in the present investigation though the exact forms of the curves differ. The titration curve has been adjusted to a scale equivalent to that of the mobility curve by making the two curves coincide at pH7 and one other pH (12.0 in this case) and then multiplying all other points on the titration curve by the factor required to make the two curves coincide at the second pH. (See the method of Abramson, Chapter VII).

ELECTROPHORETIC PATTERNS OF SODIUM DEOXYRIBONUCLEATE
(Concentration o.20%)

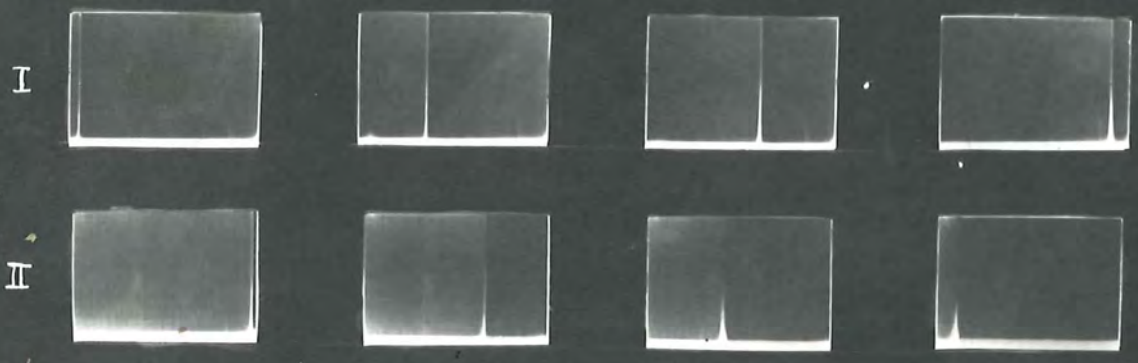
Expt.76 Acetate buffer, pH 3.98 (Ascending boundary)



Expt.93 Sodium Chloride (pH 7) I Ascending, II Descending

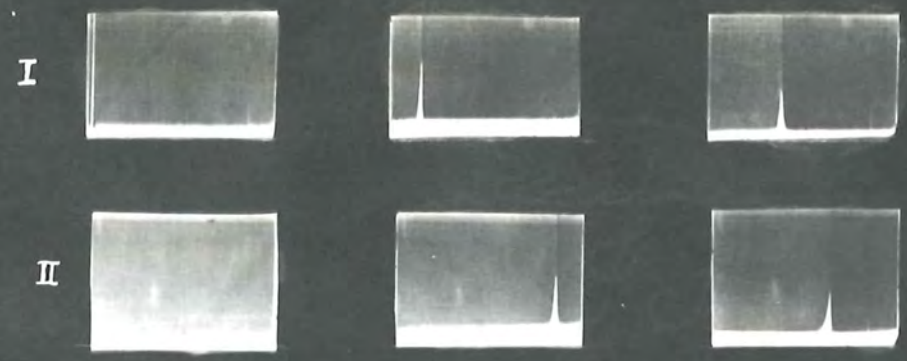


Expt.27 Carbonate buffer, pH 10.9. I Ascending, II Descending

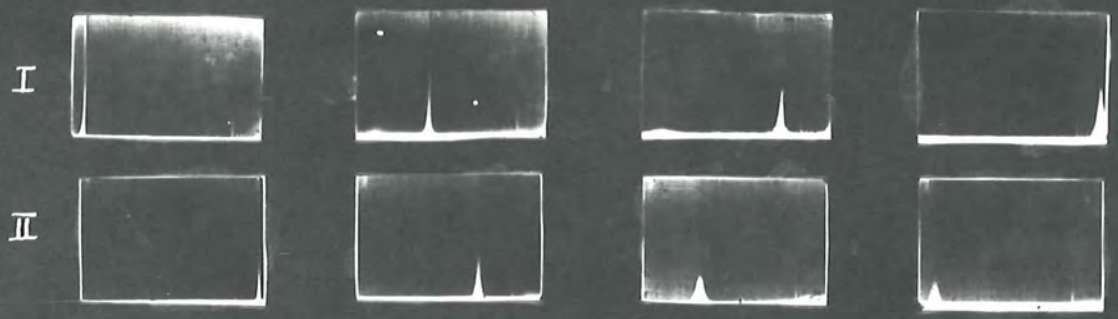


Expt 73. Phosphate buffer, pH 11.3. I Ascending, II Descending

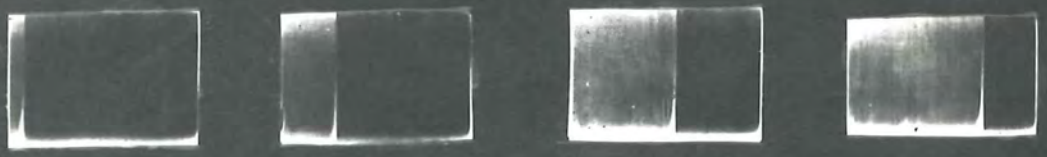
Base(+) added



Expt.62. Borate buffer, pH 6.70, after preliminary treatment with alkali to pH 12
I Ascending, II Descending



Expt.78. Carbonate buffer, pH 10.9, after preliminary treatment with alkali to pH 12
I Ascending, II Descending



Expt.102. Sodium deoxyribonucleate concentration 0.30% in 0.20 M sodium chloride (pH 7)
(Ascending boundary)

The quantitative relationship between the two curves for colloidal ampholytes has been considered by Abramson et al (11,12,9), and is discussed in the next chapter. Qualitatively, the salient features of the pH- mobility curve may be interpreted as generally conforming with the behaviour expected from the acid-base characteristics: the drop in mobility as the pH is decreased from neutrality, ascribed by Stenhagen and Teorell to decreased ionisation of the primary phosphate groups, is considered by the author, in the light of the electrometric titration evidence, to be due to the titration of the three amino groups, leading to a decrease in net charge. (Gulland, Jordan and Taylor (13)).

There is no well-marked point of inflection, as in the initial titration curve from pH7, and the mobility curve is actually more gradual than the back-titration curve. The alkaline side is similar in these respects: over the pH range 7-10, when the substance is not buffered and that part of the charge due to ionisation is constant, the mobility increases only slowly with the pH, the slope, however, being appreciably greater than that of the back-titration curve. In the region of pH 11, the slope increases, and a maximum is reached at pH 12. This behaviour is consistent with the hypothesis, indicated from viscosity and titration data, of the liberation of polynucleotide chains from the initially hydrogen-bonded aggregate, since the enolic hydroxyl groups of guanine and thymine (pKa 10.4 and 11.4

respectively) are partly ionised as they are liberated, and thus increase the total ionic charge on the particle.

This resemblance of the mobility curve to the back-titration curve rather than to the initial titration curve was enhanced by the results of experiments undertaken in order to construct a "back-mobility" curve, analogous to the back-titration curve. The mobilities of samples of the substance which had been alkali-treated and then brought back to certain pH's (in the region where the titration discrepancy occurs) fell upon the same curve as the mobilities of samples brought directly to those pH's.

This behaviour might not have been expected from first considerations, but the relationship between the mobility and the acid (base) bound is not fundamentally simple, especially in the case of non-spherical particles.

Some anomalous mobility values were observed: citrate buffers in the region pH6 gave very high mobilities (about 2μ/sec/(volt/cm.)), and borate buffers of pH9-10 gave lower values than expected (1.3-1.4μ/sec/(volt/cm.)), the latter anomaly disappearing, however, when most of the ionic strength of the buffer was made due to sodium chloride. This anomalous behaviour is probably analogous to that reported by previous investigators: Tiselius (14) noted the abnormally high mobility values of phycoerythrin in the presence of citrate, and Stenhagen and Teorell reported low mobility values for sodium

deoxyribonucleate in the presence of borate. It was pointed out in Chapter V that buffers containing highly charged ions are more liable to lead to boundary anomalies; also, Longsworth (15) has suggested that in many cases veronal buffers are most satisfactory. In the present case, several pH's were attained by the use of veronal, and the mobility values obtained were very close to those in phosphate buffers of the same pH.

The small deviation shown by the points plotted in Graph 6/2 from the smooth curve is, however, very strong evidence that, in general, the specific effects of individual buffer ions had been eliminated by working at high ionic strength, and therefore, that the curve drawn through the points represents the true characteristic mobility curve for the substance. As Longsworth (15) has shown, the deviation due to specific ion effects is liable to destroy the numerical significance of mobility values at lower ionic strengths, and it is preferable, wherever possible, to work with solutions of the same electrolyte throughout.

In addition to the qualitative interpretation above, it is now possible, owing to many theoretical investigations (those by Debye and Huckel, Henry, Abramson, Tiselius and Svenson, and Gorin being perhaps the most important) to ascribe a quantitative interpretation to the mobility data of a given system, provided the necessary colloid-chemical data are available; the relevant aspects of the theory will be considered in the next chapter.

References to Chapter VI.

(1) Stenhagen and Teorell Trans. Farad. Soc. 1939, 35, 743.

(2) Cohen J. Biol. Chem. 1942, 146, 471.

(3) Hall J. Amer . C.S. 1941, 63, 794.

(4) Seibert J. Biol. Chem. 1940, 133,593.

(5) Zittle and Seibert J. Immunol. 1942, 43, 47.

(6) Creeth, Gulland and Jordan To be published.

(7) Loring Ann. Rev. Biochem. 1944, 13, 295.

(8) Svensson, Arkiv Kem. Min. Geol. 1946, 22A, No.10.

(9) Abramson, Moyer and Gorin, "The Electrophoresis of Proteins" Reinhold 1942.

(10) Johnson Ann. Reps. 1946, 30.

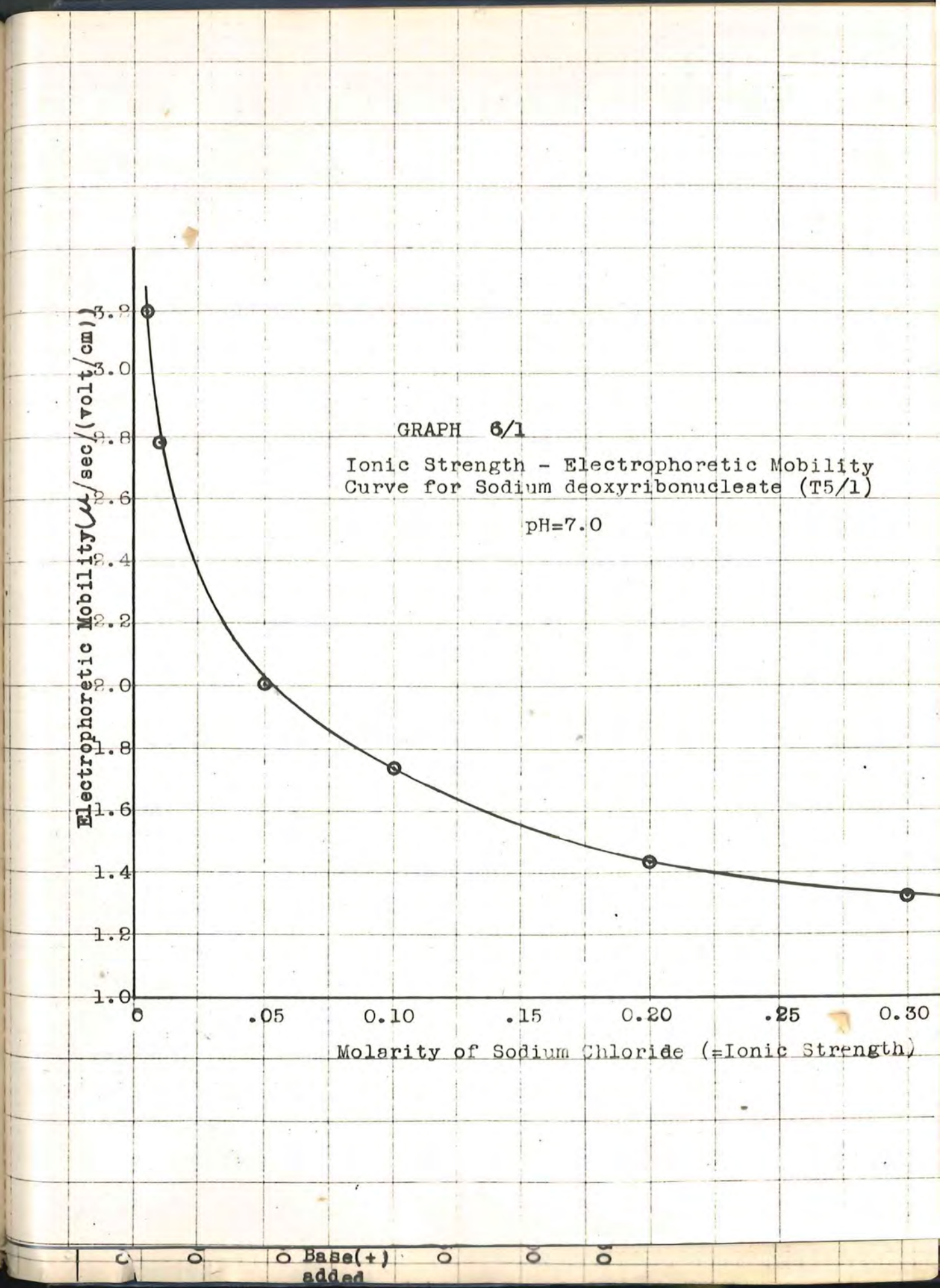
(11) Abramson J. Gen. Physiol. 1932, 15, 575.

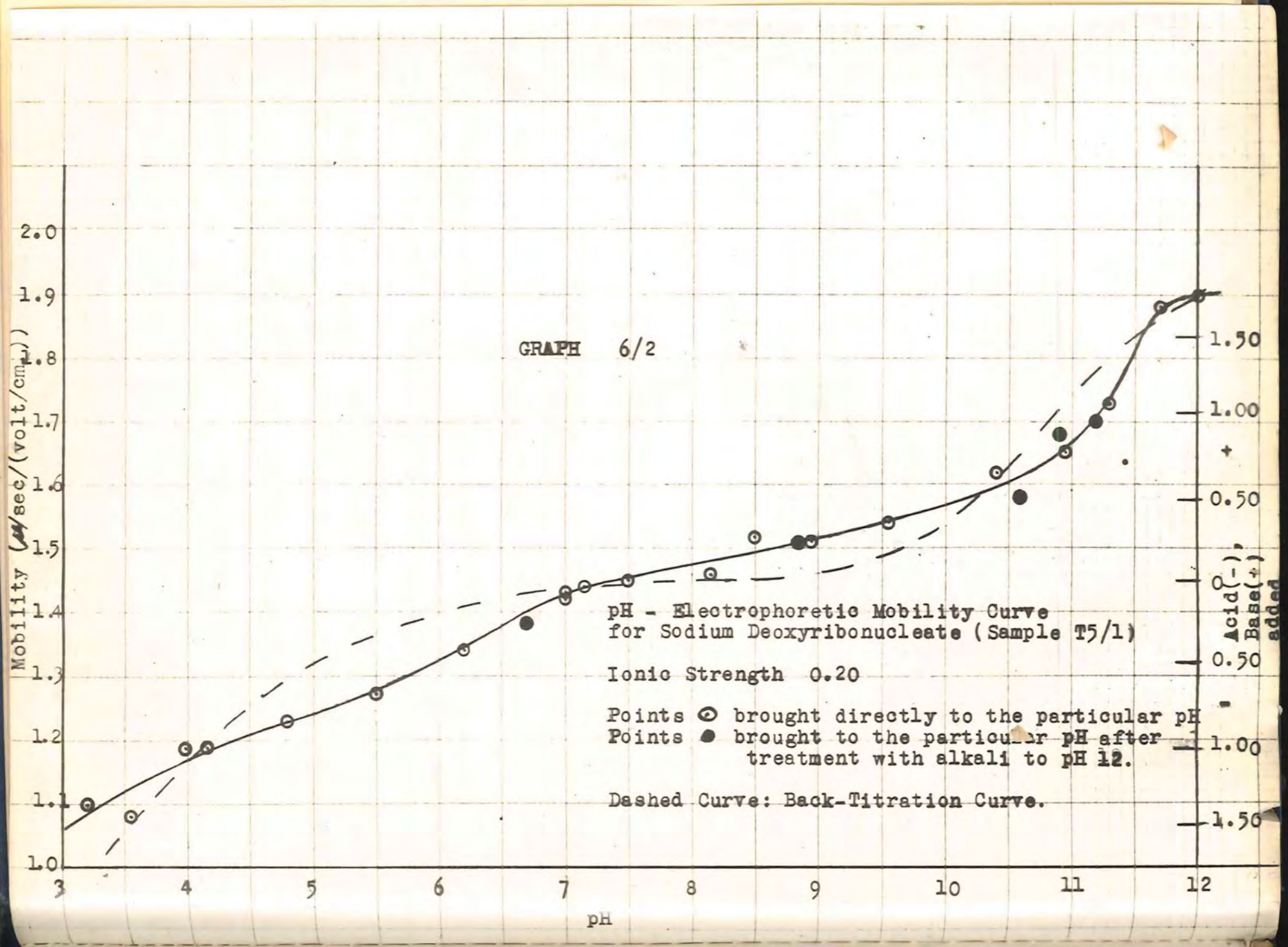
(12) Abramson, Gorin and Moyer. Chem. Rev. 1939, 24, 345.

(13) Gulland, Jordan and Taylor. J.C.S. 1947, 1131.

(14) Tiselius Nova Acta Regiae Soc. Sci. Upsaliensis,1930, 7, No.4.

(15) Longsworth Ann. New York Acad. Sci. 1941, 41, 267.


GRAPH 6/1
Ionic Strength - Electrophoretic Mobility Curve for Sodium deoxyribonucleate (T5/1)
pH=7.0
Electrophoretic Mobility (μ/sec/(volt/cm))
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
.05
0.10
.15
0.20
.25
0.30
Molarity of Sodium Chloride (=Ionic Strength)
Base(+) added


GRAPH 6/2
pH - Electrophoretic Mobility Curve
for Sodium Deoxyribonucleate (Sample T5/1)
Ionic Strength 0.20
Points ⊙ brought directly to the particular pH
Points ● brought to the particular pH after treatment with alkali to pH 12.
Dashed Curve: Back-Titration Curve.
Mobility (μ sec/(volt/cm))
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
3
4
5
6
7
8
9
10
11
12
pH
1.50
1.00
0.50
0
0.50
1.00
1.50
Acid(-), Base(+) added

Chapter VII

Theoretical Considerations, and Interpretation of Combined Data.

Section (i) The general equation of electrophoresis

(ii) Corrections to the Equation

(iii) Molecular Weight, Size and Shape Determination

(iv) Interpretation of Mobility Data

(v) The Determination of Particle Charge by Membrane Potential Measurements.

(vi) Results of Membrane Potential Measurements

(vii) Macromolecular Data - Calculation of Valences, Discussion.

- o O o -

Section (i)

The General Equation of Electrophoresis.

The viscous retarding force on a spherical particle of charge Q moving with a velocity v due to the application of an electric field X, is that given by Stokes Law. Equating the electric and viscous forces,

$QX = 6\pi\eta rv$ Where r is the radius of the particle, and η is the viscosity of the dispersion medium.

i.e. the mobility, $u = \frac{v}{X} = \frac{Q}{6\pi\eta r}$ 1

In practice, when using solutions containing other electrolytes, the mobility of charged spherical particles is always less than that given by equation 1, owing to the retardation caused by the formation round the particle of an oppositely charged ionic atmosphere. The relation between the 'zeta' potential (the potential at the surface of shear between the particle and the liquid due to to the combination of the charge on the particle and those of the ionic atmosphere) and the mobility of the particle was obtained by Smoluchowski (1) as an extension of the original theory of Helmholtz (2), by considering the case of electro-osmosis through a capillary tube. He considered the liquid within the tube to have an equal and opposite charge to that on the wall, and the charges in the liquid were assumed to be located at a fixed distance from the wall - the "rigid doubtful layer" of Helmholtz.

Smoluchowski's final equation was:-

$$u = \frac{\zeta D}{4\pi\eta} \qquad 2.$$

Where D is the dielectric constant of the liquid.

This relates the mobility to the zeta potential in the case where the relative motion is at all times parallel to the direction of the double layer: it will thus apply to the motion of liquid in a capillary, and to the motion of a long cylindrical particle migrating parallel to the direction of the applied electric field; it will not apply, however, to a small spherical particle.

For a large sphere of radius r and charge +Q, separated by a distance d from its attendant rigid double layer of charge -Q, the zeta potential is (by definition):-

$$\zeta = \frac{Q}{Dr} - \frac{Q}{D(r+d)} = \frac{Qd}{Dr(r+d)} \qquad 3.$$

In practice, however, as Gouy (3) pointed out, the double layer is diffuse, a region, or ionic atmosphere which will contain, over a time average, statistically more ions with a charge opposite to that of the particle than with the similar charge.

The relation between the zeta potential and the concentration of other ions in the solution was first solved by Gouy (3), but is more conveniently expressed in the terms of the well-known Debye - Huckel theory (4) of strong electrolytes.

Considering the case of a large positively charged particle in a solution of potassium chloride, if the potential due to the charged surface and its attendant ionic atmosphere be ψ ,

then the energy released when a chloride ion (charge -e) is brought from a point of zero potential to the point of potential ψ is $+e\psi$, and the energy released when a similar motion of the potassium ion is caused will be $-e\psi$. Then applying Boltzmann's principle to the distribution of K^+ and Cl^- ions in the neighbourhood of the particle, the number of positive ions n_+ in an element of volume dv will be:-

$$n_+ = n\varepsilon^{-e\psi/kT} . dv$$

where n is the total number of ions per c.c., k the Boltzmann constant, and T the absolute temperature.

A similar equation holds for the number of negative ions, so the charge density ρ about the point of potential ψ willbe:-

$$\rho = (n_+ + n_-)\, e = ne\varepsilon^{-e\psi/kT} - ne\varepsilon^{+e\psi/kT}.$$

$$= 2ne \sinh \frac{e\psi}{kT} \qquad 4.$$

Also ψ and ρ must be related by Poisson's equation:-

$$\frac{d^2\psi}{dx^2} + \frac{d^2\psi}{dy^2} + \frac{d^2\psi}{dz^2} = \nabla^2\psi = -\frac{4\pi\rho}{D}$$

Therefore $$\nabla^2\psi = \frac{8\pi ne}{D} \sinh \frac{e\psi}{kT} \qquad 5.$$

If the electric energy $e\psi$ is small compared with the thermal energy kT, then the sinh becomes equal to the argument, and

$$\nabla^2\psi = \frac{8\pi ne^2}{DkT}\psi \qquad 6.$$

or $$\nabla^2\psi = K^2\psi \text{ , where } K = \sqrt{\frac{8\pi ne}{DkT}}^2 \qquad 6.$$

In the general case, where ions of valency $Z_{i,j}$ are present

in concentrations $c_{i,j}$, etc.

$$K = \sqrt{\frac{4\pi Ne^2}{1000DkT}} \cdot \sqrt{\Sigma c_{i,j} z_{i,j}^2} \qquad 7.$$

In water at 0°, $K = 0.323 \times 10^8 \sqrt{\mu}$, where μ is the ionic strength function of Lewis and Randall ($\mu = \frac{1}{2}\Sigma c_{i,j} z_{i,j}^2$). Equation 7 was evolved without any assumption as to particle shape or size, and so applies to all electrokinetic phenomena.

For a sphere of charge Q and radius r, ψ is a function only of x, the radial distance from the centre of the sphere, and the Debye - Huckel equation becomes:-

$$\frac{d^2\psi}{dx^2} + \frac{2}{x}\frac{d\psi}{dx} = K^2\psi \qquad 8.$$

When $x = \infty$, $\psi = 0$, and when $x = r$, $\left(\frac{d\psi}{dx}\right) = -\frac{Q}{Dr^2}$.

(The latter boundary condition follows from the definition of ψ:-

$$\psi = \frac{Q}{Dr} + \psi'$$

the first term is the potential due to the charge on the sphere, and the second is that due to the charges of the ionic atmosphere.

Then $$\left(\frac{d\psi}{dx}\right)_{x=r} = -\frac{Q}{Dr^2} + \frac{d\psi'}{dr}$$

At the surface of the sphere ($x = r$), ψ' is constant, therefore $\frac{d\psi'}{dr} = 0$).

The solution of equation 8 with these boundary conditions is then:-

$$\psi = \frac{Q\, e^{-K(x-r)}}{Dx(1+Kr)} \qquad 9.$$

This equation gives the potential drop through the double layer.

Since ζ is defined as and the value of ψ at x = r

$$\zeta = \frac{Q}{Dr} \cdot \frac{1}{1+Kr} \qquad 10.$$

(This equation may be written $\zeta = \frac{Q}{Dr} \cdot \frac{1/K}{(1/K)+r}$, which may be compared with 3: it is evident that $\left(\frac{1}{K}\right)$, which has the dimensions of length, is equal to the distance d of the equivalent rigid double layer).

Equation 10 thus relates the zeta potential of a charged sphere to the concentrations of other ions present in solution, For a large sphere (or any surface of small curvature and easy shape):-

$$u = \frac{\zeta D}{4\pi\eta} \quad \text{(Equation 2)}$$

and accordingly, from equation 10:-

$$u = \frac{Q}{4\pi\eta r} \cdot \frac{1}{(1+Kr)} \qquad 11.$$

For a small sphere (from equation 1, by analogy with equation 2):-

$$u = \frac{\zeta D}{6\pi\eta}$$

Therefore $$u = \frac{Q}{6\pi\eta r} \cdot \frac{1}{(1+Kr)} \qquad 12$$

the equation of Hückel (5).

The question of which of these two equations 11 and 12 actually described the motion of a given particle was finally settled by Henry (6), after some confusion. Henry considered the distortion of the external field produced by the charges of the double layer, and the effect of this upon the viscous forces.

Gorin (7) slightly modified Henry's equations, and the final result obtained is the general equation:-

$$u = \frac{\zeta D}{6\pi\eta} . f(Kr) \qquad 13.$$

where f(Kr) is the Henry function:-

$$f(Kr) = 1 + \frac{1}{16}(Kr)^2 - \frac{5}{48}(Kr)^3 - \frac{1}{96}(Kr)^4 + \frac{1}{96}(Kr)^5 - \left[e^{Kr} \cdot \left(\frac{12}{96}(Kr)^4 - \frac{1}{96}(Kr)^6\right) \int_{\infty}^{Kr} \left(\frac{e^{-t}}{t} . dt\right)\right] .$$

Gorin (7) has tabulated values of this function for different values of (Kr): the function varies between a value of 1.00 at low values of (Kr) (i.e. the case of the small sphere), so that equation 13 becomes identical with equation 12, and a value of 1.5 at very large (Kr) values - so that equation 13 reduces to equation 11.

Accordingly the difference between these equations is satisfactorily resolved; the final equation for the electrophoresis of a spherical particle then becomes:-

$$u = \frac{Q}{6\pi\eta r} . \frac{f(Kr)}{1+Kr} \qquad 14$$

Using this equation, the mobility of spherical particles of any size may be predicted if the charge and radius are known (8).

This has so far only been done by Tiselius and Svensson (9), for the protein ovalbumen, values of the charge being obtained from Adair and Adair's (10) work on membrane potentials (see below, Section (v)), the molecular weight and hence the

radius being known from Svedberg's sedimentation data. They predicted the theoretical mobility at various ionic strengths, and obtained very good agreement with experimental results. It thus appeared that the various models employed in the theoretical development of sedimentation, membrane potential, and electrokinetic phenomena were very close to the actual conditions encountered by the molecular particle.

However, this excellent agreement was somewhat fortuitous, as has been pointed out by Gorin (7), as Tiselius and Svensson did not take into account the various other corrections which must theoretically be applied. These are treated in the next section.

Section (ii)

Corrections to the Simple Equation of Electrophoresis.

(a) The first correction that must be considered is that due to the finite size of the ions in the ionic atmosphere; it was first developed and used by Gorin (11).

In the derivation of equation 14 it was assumed that the surface of shear was identical with the shell of nearest ions of the atmosphere. Since these ions behave (as do all symmetrically charged spheres) as if their charge were concentrated at the centre, the actual nearest charged shell is situated at a distance $(r+r_i)$ from the centre of the migrating particle, where r_i is the average radius of the ions in the ionic atmosphere. Consequently, the boundary condition

that $\frac{d\psi}{dx} = \frac{Q}{Dr^2}$ at $x = r$ is modified to

$$\left(\frac{d\psi}{dx}\right) x = r + r_i = \frac{Q}{D(r+r_i)^2}$$

Whence the final equation of electrophoresis of spheres becomes $u = \frac{Q}{6\pi\eta r} \cdot \frac{f(\kappa r).(1+\kappa r_i)}{(1+\kappa r + \kappa r_i)}$ 15

This equation differs from the uncorrected form 14 mainly in the numerator where the new term $(1 + \kappa r_i)$ is introduced: this may cause a mobility difference of as much as 20% at the higher ionic strengths.

(b) The Valence Effect.

As Gorin (7) points out, the assumption in the Debye - Huckel derivation of the Zeta potential that the electric energy $e\zeta$ is small compared with the thermal energy kT can only hold at low values of ζ ; deviations must accordingly be expected if the charge on the particle is high or if the radius is small.

In this case, the differential equation of the diffuse double layer (of univalent ions) surrounding a spherical particle, which has the exact form:

$$\nabla^2\psi = \frac{8\pi ne}{D} \sinh \frac{e\psi}{kT}$$, has not been completely solved: approximate solutions are, however, available from the work of Gronwall, La Mer, and Sandved (12).

The effect of the variation between sinh and argument is to reduce the mobility of a highly charged particle below that value predicted by the corrected form of the Debye - Huckel

equation 15; an attempt has been made by Gorin (7,13,15) to calculate the magnitude of this correction, which he formulates as a modified form of Debye - Huckel equation:

$$u = \frac{Q \cdot f(Kr)}{6\pi\eta r} \cdot \left(\frac{1 + Kr_i}{1+Kr+Kr_i} - n \right) \qquad 16$$

where n, the correction term due to the valence effect, is given by

$$n = 6 \left(\frac{r}{r+r_i} \right) \left(\frac{ze^2}{D(r+r_i)kT} \right)^2 \left[\tfrac{1}{2} X_3(Kr+Kr_i) - 2Y_3(Kr+Kr_i) \right] \qquad 17$$

In this equation, z is the valence (the number of excess positive or negative charges per molecule), and X_3 and Y_3 are functions of $(Kr+Kr_i)$ which were derived by Gronwall et al and are available in tabular form for various $K(r+r_i)$ values in their original paper (12), and also in the work of Harned and Owen (14). The complete derivation of equation 17 has not yet been published, however, and at present it is not desirable, in the author's opinion, to apply large correction factors in this manner.

(c) The Asymmetry Effect.

The two general factors modifying the simple Debye - Huckel equation have now been considered; the special case of the cylindrical particle to which model the deoxyribonucleate ion most closely approximates, must now be examined.

The Debye - Huckel equation for a cylinder has the form

$$\frac{d^2\psi}{dx^2} + \frac{1}{x}\frac{d\psi}{dx} - K^2\psi = 0 \qquad 18$$

This is a modified Bessel's equation of zero order, and was

solved by Gorin (8) by making the usual assumptions of boundary conditions and uniformity of charge distribution.

The final equation for the zeta potential then becomes

$$\zeta = \frac{2\,Q}{D(1+2a)} \cdot \left[\frac{K_o(Ka+Kr_i)}{(Ka+Kr_i)\cdot K_1\,(Ka+Kr_i)} + \ln\left(\frac{a+r_i}{a}\right)\right] \qquad 19$$

where l is the length and a the radius, of the cylinder ($l \gg a$), and K_o and K_1 are Bessel functions, which have been given in tabular form for various $(Ka+Kr_i)$ values in the work of Abramson et al (13).

The relation between the zeta potential and the mobility is not as simple as for the case of the spherical particle, as the force exerted by the field on the particle will vary with the orientation of the latter: i.e. the factor C in the general equation of electrophoresis

$$u = C\frac{\zeta D}{\pi\eta}$$

has different values for differantly oriented particles. For those lying in the direction of the field: $C = \frac{1}{4}$ so that the mobility is that given by the Smoluchowski equation 2 for electro-osmosis and the general equation for the large particle, where the field is at all times parallel to the surface.

For particles oriented perpendicular to the field, the distortion of the field by the double layer will depend on Ka ; accordingly $C = \frac{1}{F(Ka)}$. The function $F(Ka)$ for the perpendicular cylinder was obtained graphically by Gorin (16) by an extension of the work of Henry (6)

For the practical case of randomly oriented cylinders, there are, on the average, two perpendicular cylinders to each parallel to the field, so that the C factor becomes

$$C = \frac{3}{4+2F(Ka)}$$

i.e. $C = \frac{1}{F'(Ka)}$ where $F'(Ka) = \frac{2F(Ka)+4}{3}$ (Gorin). 20

Abramson et al (13) have given tables of F(Ka) and F'(Ka) for various (Ka) values. Accordingly, the general equation for the electrophoresis of cylindrical particles then becomes

$$u = \frac{\zeta D}{F'(Ka)\pi\eta} \quad ,$$

and incorporating the value of the zeta potential from equation19

$$u = \frac{2Q}{(1+2a)\cdot F'(Ka)\cdot\pi\eta} \cdot \left[\frac{K_0(Ka+Kr_i)}{(Ka+Kr_i)\cdot K_1(Ka+Kr_i)} + \ln\left(\frac{a+r_i}{a}\right) \right] \quad 21$$

This equation represents adequately the behaviour of cylindrical particles of widely differing size, provided the zeta potentials are low. The application of a correction for highly charged cylinders, corresponding to that developed by Gorin for spheres is not at present possible. It must also be emphasised that no correction (for spheres or cylinders) is available for the effect of 'relaxation phenomena' i.e. the asymmetry of the ionic atmosphere in relation to the particle, due to the movement of the latter under the applied field.

To summarise, therefore, the present state of the theory

of electrokinetic migration is such as to enable a fairly complete visualisation to be made of the migrating particle, if the various macromolecular data are available; these are (a) the electrophoretic mobilities under various conditions, (b) the molecular weight and density, and (c) the axial ratio, if the particle is not spherical. From (b) and (c) the particle dimensions may be calculated.

With this information, the charge on the particle may be calculated from the mobility and compared with the value obtained from titration data, or alternatively, if some other means of determining the charge are available, the mobility of the particle may be calculated, using the appropriate equation, and compared with the experimental values.

Before discussing these methods of interpretation, it is appropriate to consider very briefly the second essential factor which must be known, i.e. the particle dimensions.

Section (iii).

Molecular Weight, Size and Shape Determination.

The particle dimensions are generally calculated from molecular weight and shape data which, owing to the developement of the ultracentrifuge by Svedberg et al (17), are now available for many colloids, particularly the proteins. The established procedure is to determine the sedimentation constant (S) and the diffusion constant (D), (the latter generally by the method of Lamm (18)).

By applying the equation $M=\frac{RTs}{(1-\rho\bar{V})D}$, 23

(where ρ is the density of the solution, and $\bar{V}$ the partial specific volume of the colloid) an absolute value of the molecular weight (independent of shape or size) is obtained. The diffusion constant for a spherical particle of this molecular weight may then be calculated from the Stokes - Einstein relationship:

$$D_o = \frac{kT}{6\pi\eta r} \quad , \qquad 24$$

where r is the radius of the particle, of density ρ', calculated from the equation

$$\frac{4\pi r^3 \rho'}{3} = \frac{M}{N} \cdot$$

The ratio of this value D_o to D, the experimentally determined diffusion constant, is equal to the 'frictional ratio' f/f_o, the ratio of the molar frictional constants, (f_o corresponding to the sphere), which is related to the axial ratio of the particle by the equation of Perrin (19) for oblate ellipsoids:

$$D_o/D = f/f_o = \frac{(1-k^2)^{\frac{1}{2}}}{k^{2/3} \ln\left(\frac{1+(1-k^2)}{k}\right)} \qquad 25$$

where $k = \frac{b}{a}$, b being the length of the equatorial axis of the ellipsoid, and a the length of the axis of revolution i.e. for cylindrical rods, $\frac{a}{b}$ is the axial ratio.

If $\frac{D_o}{D}$ differs little from unity, the particle is assumed to be spherical, the radius may be calculated: if the ratio is appreciably greater than unity, the dimensions of the cylinder of appropriate axial ratio may be obtained.

If ultracentrifugal information is not available, viscosity and diffusion data may be combined in a similar manner: subject to the conditions discussed in Chapter II, it is often possible to calculate the axial ratio from the Simha equation (p21), and thus to calculate the dimensions of a cylinder of appropriate diffusion constant.

However, the question is complicated by the presence, in most cases, of bound water of hydration, the effect of which is, on these premises, indistinguishable from asymmetry. Thus the radius calculated from diffusion data corresponds to the hydrated sphere, whereas that from sedimentation velocity corresponds to an unhydrated sphere, since the partial specific volume $\bar{v}$ in the sedimentation equation refers to the dry material. Accordingly the ratio $\frac{D_o}{D}$ may correspond to a certain value of the asymmetry of unhydrated particles, or to the presence of a spherical particle with a certain amount of hydration, or probably to a combination of both factors.

This question has been extensively surveyed by Oncley et al (20,21);as Oncley suggests, if the possible values of the asymmetry (assuming no hydration) are plotted graphically against the possible values of the hydration (assuming spherical particles) allowing certain probable experimental errors for both, then the areas, corresponding to a certain value of the asymmetry plus some hydration, which are common to the different experimental methods which will give the

most probable model of the actual system.

For systems of well-defined sedimentation and diffusion behaviour, where the charge on the particle is also known with a high degree of certainty, the mobility characteristics enable some choice to be made between the asymmetry and the hydration (Gorin (13, 16)): by applying the equations for a sphere, or cylinders of various axial ratios and choosing that which gives the closest fit, some idea of the relative magnitude of these factors may be obtained, depending, of course, on the accuracy with which the charge is known.

Section (iv)

Interpretation of Mobility Data

With molecular dimensions available, the quantitative interpretation of mobility data becomes possible.

Calculation of Charge from Mobility

The general relationship between the titration curve and the pH mobility curve for proteins has been largely investigated, notably by Abramson et al (22, 8, 13) who formulated the proposition that, subject to certain conditions, the mobility in uni-univalent eletrolytes of constant ionic strength was directly proportional to the amount of acid (base) bound. The essential conditions were that (i) the protein salts were always completely dissociated, (ii) the amount of ions other than H_3^+O or OH^- bound was independent of pH, and (iii) the protein did not alter in size and shape at different pHs.

It is evident that this statement follows from the general equation of electrophoresis of spherical particles:-

$$u = \frac{Q}{6\pi\eta r} f(Kr) \cdot \frac{(1 + Kr_i)}{(1 + Kr + Kr_i)} ,$$

subject to these conditions.

Thus in the ideal case, the mobility variation is determined solely by the charge variation, and the latter is directly proportional to the acid (base) bound: accordingly the mobility - pH curve should coincide completely with the titration curve if (a) the latter is shifted along the axis representing hydrogen ions bound so that its zero point is identical with the isoelectric point from mobility measurements, and (b) if all the points on the mobility curve are multiplied by that factor which would make any one point on the mobility curve agree with the titration curve at that pH.

For many proteins, notably ovalbumen, β lactoglobulin, and gelatin, very good agreement between the two curves was obtained, and it was consequently assumed that the net charge on the particle calculated from the acid (base) bound at a certain pH and the value calculated from the mobility at that pH, should give similar values.

Serious discrepancies were at first observed, however, as neither the possibility of the specific binding action of neutral salts, nor the possible asymmetry had been taken into account.

The procedure was further developed by Gorin and Moyer (16) for the case of serum albumen B. The most probable value of

the asymmetry of
the particles was calculated from other data, and this model used to provide the quantities in the general equation for the electrophoresis of cylinders (equation 21, Page 164). The charge was then calculated from the mobility, and plotted against the pH. These investigators also found it necessary to correct the titration data, which were extrapolated to zero protein and electrolyte concentrations (the omission of this correction may lead to errors of several hundred per cent). When the adjusted and corrected titration curve was plotted on the same graph as the charge, almost complete coincidence was observed, thus showing that the models used in the determination of the charge approximated closely to the actual conditions.

In the present investigation, this method has not been followed; preliminary calculations of the charge from mobility data shewed large discrepancies from that expected from the titration curve. Furthermore, the osmotic pressure determinations of Hammarsten (23) and Greenstein (24) indicated thatin the viscous solutions of the sodium deoxyribo-nucleate, many of the sodium ions were osmotically inactive, a phenomenon which has been called the Hammarsten effect (25). If, as these investigators assumed, the sodium ions are bound within the micelle, then the apparent charge might well be reduced.

Therefore, it seemed desirable to follow the second method of interpretation of mobility data, and to obtain independent values of the charge on the particle, so that theoretical mobilities might be calculated and compared with those observed,

at the different ionic strengths. This procedure is probably the best method of testing the validity of the mobility equations.

Section (v)

The Determination of Particle Charge by Membrane Potential Measurements

This method, which was developed by Loeb (26), Bjerrum (27), Northrop and Kunitz (28), and brought to a greater degree of accuracy by Adair and Adair (29, 10), is well suited to the present purpose: the investigation of the well known Donnan equilibrium (30), in which the presence of a colloid causes the unequal distribution of electrolyte on the two sides ofa membrane impermeable to the colloid ion, may yield values of the equivalent concentration of the colloid.

The following treatment is derived partly from Adair and Adair's work (29) but differs in some aspects, and is accordingly given in full.

If a solution of sodium chloride, of equivalent concentration c_1 containing the sodium salt of a colloid, equivalent concentration C_2 is separated by a membrane (permeable to sodium and chloride ions, but impermeable to the colloid ion) from an outer solution of sodium chloride, also concentration c_1, then some sodium chloride diffuses out of the membrane, in orderthat the chemical potential of the substances common to both sides of the membrane should be equal, which is the fundamental requirement of the Donnan equilibrium.

The chemical potential μ of a salt is defined as the sum of the potentials of its ions; accordingly, at equilibrium:-

$$\mu^{o}_{Na} + RT \ln a_{Na(1)} + \mu^{o}_{Cl} + RT \ln a_{Cl(1)} = \mu^{o}_{Na} + RT \ln a_{Na(2)} + \mu^{o}_{Cl} + RT \ln a_{Cl(2)} \quad \ldots 1$$

The subscripts 1 and 2 refer to the activities of the ions in the outer and in the inner solutions respectively.

Therefore $a_{Na(1)} \times a_{Cl(1)} = a_{Na(2)} \times a_{Cl(2)}$ 2

In fairly dilute electrolyte solutions, and low concentrations of colloid, the activity coefficients of the ions will not vary appreciably across the membrane, and the activity terms may be replaced by concentrations.

If the system is:-

Na^+	Cl^-	:	Na^+	R^-	Na^+	Cl^-
C_1	C_1	:	C_2	C_2	C_1	C_1

at the commencement of dialysis, then at equilibrium an amount of sodium chloride x (in equivalent concentration terms) will have diffused out of the membrane into the dialysate.

The equilibrium concentrations then become:-

Na^+	Cl^-	:	Na^+	R^-	Na^+	Cl^-
$(C_1 + x\frac{v_2}{v_1})$	$(C_1 + x\frac{v_2}{v_1})$	:	C_2	C_2	(C_1-x)	(C_1-x)

and from equation 2:-

$$\left(C_1 + \left(x\frac{v_2}{v_1}\right)\right)^2 = (C_1 - x + C_2)(C_1 - x) \qquad 3$$

where v_1 and v_2 are the volumes of the outer and inner solutions respectively.

From equation 2 (in concentration terms):-

$$\frac{[Na]_2}{[Na]_1} = \frac{[Cl]_1}{[Cl]_2} = f \qquad 4$$

where f is the ideal distribution ratio, applying to all diffusible univalent ions.

Therefore, for sodium ions:-

$$f = \frac{(C_2 + C_1 - x)}{\left(C_1 + x\left(\frac{V_2}{V_1}\right)\right)}$$

The equivalent concentration of colloid ions is then:-

$$C_2 = f\left(C_1 + x\frac{V_2}{V_1}\right) - (C_1 - x)$$
$$= [Na]_2 - [Cl]_2 \qquad 5$$

This is a particular case of the general expression:-

C_2 = Sum of equivalent concentrations of cat ions − Sum of equivalent concentrations of anions

Also: "Valence" = $\frac{\text{Equivalent Concentration}}{\text{Molecular Concentration)}}$,

so that the charge on the particle may be determined (if the molecular weight is known) from the concentrations of diffusible ions within the membrane.

These concentrations may be determined analytically, or by employing the system as a concentration cell: the presence of differing concentrations of the same ions on the two sides of the membrane gives rise to a potential across the membrane, the magnitude of which may be measured by suitable methods. The potential E is given by Nernst's equation:-

$E = -\frac{RT}{nF} \ln \frac{a(1)}{a(2)}$ for each ion, where n is the valence of the ion of activity a.

In this case:-

$$E = +\frac{RT}{F} \ln \frac{[Na]_2}{[Na]_1} = +\frac{RT}{F} \ln \frac{[Cl]_1}{[Cl]_2} = + \frac{RT}{F} \ln f. \qquad 6$$

Thus f, the distribution ratio may be determined from the membrane potential; since $[Na]_2 = f[Na]_1$, and $[Cl]_2 = [Cl]_1/f$, from equation 4, then equation 5 may be re-written:-

$$C_2 = f[Na]_1 - [Cl]_1/f, \qquad 7$$

so that the valence may be determined from the concentrations in the dialysate.

This equation, 7, was derived for dialysis to equilibrium without replacement of the outer solution; since, however, it is only the ratio of the concentrations which determines the membrane potential (equation 6), and the ratio will be finally unaltered by replacement, the equation may also be used forthe case of dialysis with constant replacement of the outer solution, until further change produces no alteration in the membrane potential. This latter process is much to be preferred, as any diffusible impurities present originally in the inner solution are removed, as are also any traces of electrolyte introduced during a measurement. Furthermore, the final ionic concentrations of the outer solution are already known with great accuracy. For equilibration in solutions containing divalent anions, the modified form of equation 7 holds; for example, in phosphate buffers:-

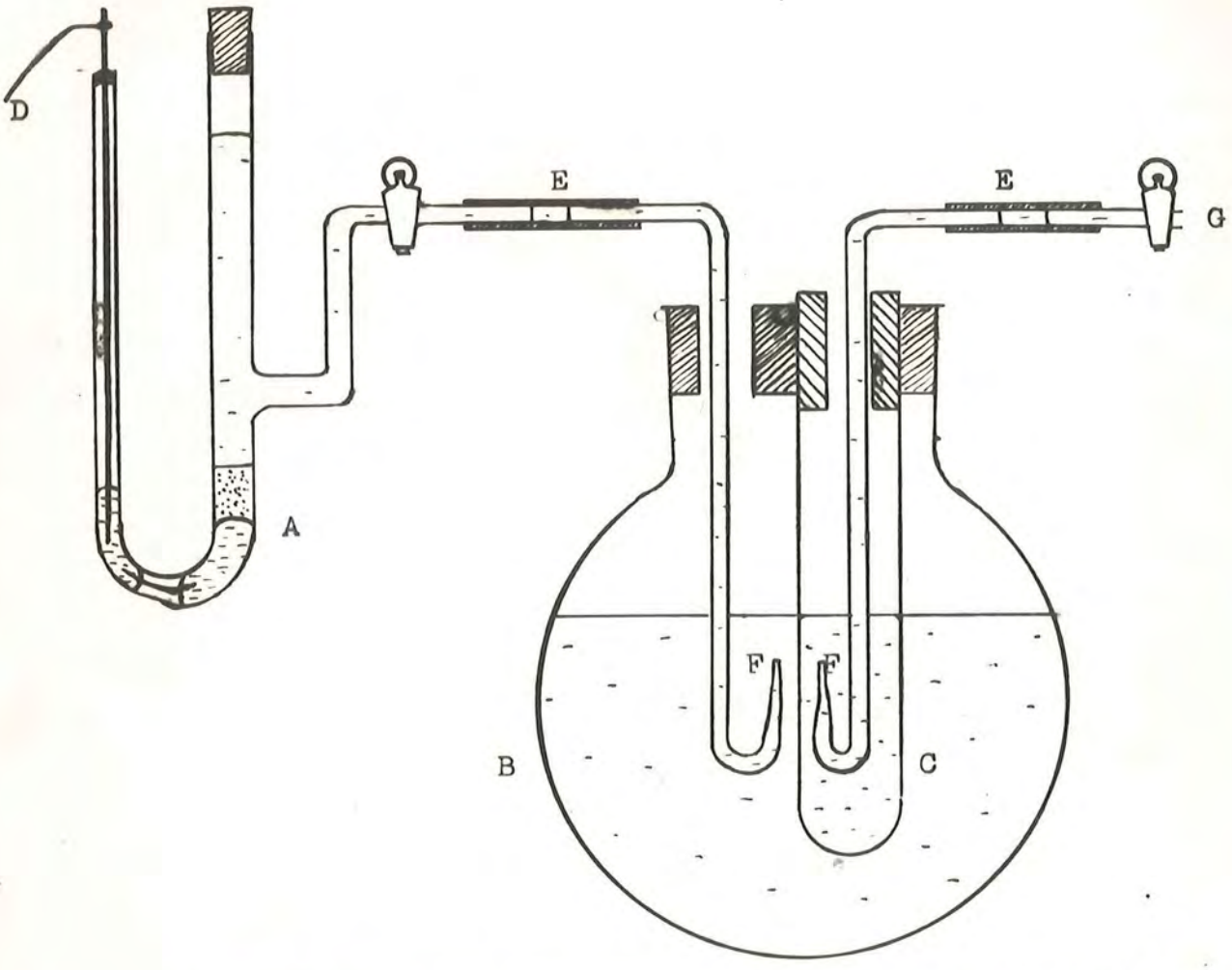
$$C_2 = f[Na]_1 - \left([H_2PO_4^-]_1/f\right) - \left([HPO_4^{=}]_1/f'\right)^1 ,$$

where f' is given by $E = \frac{RT}{2F} \ln f'$.

The Measurement of Membrane Potentials

Adair and Adair (29) have surveyed very thoroughly the errors inherent in the various methods of determining the activities of an ion at two different concentrations separated by a membrane, but point out that if the potentials are kept low, the ideal relations must apply. Since the valences of the proteins they investigated were low (~10), they were forced to use high concentrations (~20%) of protein, when corrections must be applied for the bulk of colloid present.

In the present case, fairly low concentrations of sodium deoxyribonucleate (<1%) were used, and the corrections are vanishingly small, and were neglected. An apparatus similar to that described by Adair was used, and is illustrated on Page176: two calomel cells (later, silver-silver chloride cells were found preferable) with up-turned tips are employed, one dipping into the colloid solution contained in a collodion membrane, and one into the dialysate. The EMF of the system then depends on the relative concentrations of the ions within and without the membrane and on the two liquid junctions involved. As Stadie and Hawes (31) have shewn, the liquid junctions formed in the capillary tips of the calomel cells, between the relatively concentrated potassium chloride solution and buffer, or colloid-buffer, solutions are reproducible, and largely eliminate the difference in liquid junction potentials at the two boundaries. The actual potential across the membrane was shewn by Adair and Adair (for protein solutions) to be inde-



APPARATUS FOR THE DETERMINATION OF MEMBRANE POTENTIALS

A. First calomel cell (positive in this case)
B. Wide-necked flask, containing dialysate
C. Collodion membrane, containing nucleate solution
D. Lead to potentiometer
E. Rubber joining pieces
F. Upturned capillary tips
G. Second calomel cell (negative in this case)

pendent of the chemical nature of the membrane, the potentials across collodion, parchment, and cellophane, being nearly identical at comparable time intervals. All equilibrations were carried out at 0.5°C, when the possibility of changes in the colloid material due to bacterial action is greatly diminished.

In view of the small magnitudes of the membrane potentials, and the errors inherent in the method, the results obtained cannot be of great accuracy. As Adair and Adair point out, however, the great interest attaching to the application of even approximate results renders the method of considerable value.

The experimental details of the preparation of the membranes and solutions, and the technique of EMF measurements are described in Chapter VIII.

Section (vi)

Results of Membrane Potential Measurements

The valence of the deoxyribonucleate ion was determined at a series of ionic strengths, some in sodium chloride alone, and some in (sodium) phosphate buffers of pH 7.0. In agreement with expectation, the apparent valences of the substance in these media were of similar magnitude.

The results obtained are shewn in Table I. (The tabulated record of experimental results is shown on Page 198). The values were calculated from the equivalent concentrations using the figure of 8.2×10^5 for the molecular weight, obtained by Cecil and Ogston (see Section (Vii). It is evident that at

all ionic strengths the valence is much less than the theoretical value of 2,664 calculated from the analytical data, assuming a polytetranucleotide structure, $\left[(C_{39}H_{45}O_{24}N_{15}P_4)\right]_{366}^{-} = 8.2 \times 10^5$.

Table I

The Apparent Valences of the Deoxyribonucleate Ion at pH 7.0.

Electrolyte Solution	Ionic Strength	Apparent Valence.
Sodium Chloride	0.005	509
Phosphate	0.01	508
Sodium Chloride	0.02	615
Phosphate	0.05	698
Sodium Chloride	0.08	882
Sodium Chloride	0.20	830, 1,090, 1,100.

The occurrence of rather large discrepancies between the observed and calculated valences is in qualitative agreement with the behaviour of solutions of this material reported by Hammarsten (23): he observed that the osmotic pressures were much lower than expected from the electrical conductivities, though they agreed with the values expected from freezing point methods. He ascribed the phenomenon to the 'enmeshing' of some of the sodium ions by the colloidal micelle, so that they were osmotically inactive. Linderstrom-Lang (32), however, preferred to attribute the Hammarsten effect to the considerable deviations from ideal solution laws which must be expected to occur in systems of dynamically interdependent molecules. A similar explanation was advanced by Van Ryssel-

berghe (33), on the basis of Debye-Huckel interaction, and recently Mukherjee and Sarkar (34) have postulated some form of particle aggregation to account for allied discrepancies in the behaviour of solutions of yeast ribonucleic acid. Adair (35), considering the similar but less well-marked effect in the case of some proteins, attributed it, in part, to the binding of electrolytic ions (of opposite charge) to the colloidal particle, and he shewed by membrane potential experiments and analyses, the magnitude of this effect, which was termed the 'surface excess' of opposite ions.

It may also be noted that the case of gum arabic is completely analogous. Here, the discrepancy between observed and calculated valences (Svensson (36)) was similarly attributed to 'ion-pair' interaction between cations and the carboxyl groups of the gum molecule.

It is probable, therefore, that some combination of the effects of particle interaction and adsorption of ions of opposite sign is the cause of the discrepancy in the present instance, and also of the rather large variation of the valence with the ionic strength.

In view, however, of the present uncertainty as to the precise nature of these effects and the rather low degree of accuracy at present obtainable in membrane potential experiments, the interpretation of the results can at best be semi-quantitative. The following application of the apparent valence values must accordingly be considered as demonstrating the possibilities

of a promising method of approach to the problem, rather than as a complete exposition of the many factors determining the electrophoretic mobility.

Section (vii)

Macromolecular Data, calculation of Mobility, Discussion.

The conclusions from the viscosimetric evidence described earlier that the particles of sodium deoxyribonucleate were long, very asymmetric, and closely associated with each other, have been confirmed by the results of the ultracentrifugal sedimentation experiments of Cecil and Ogston (37). These investigators were using the same sample of material.

The sedimentation constant varied somewhat with the concentration but it was concluded from the shape of the sedimenting boundaries that the sample consisted of a simple homogeneous component, shewing that the material was monodisperse with regard to molecular weight. These findings, combined with the homogeneous electrophoretic migration under all conditions, constitute strong evidence for a very high degree of molecular homogeneity.

By extrapolation to infinite dilution of colloid concentration the value of the sedimentation constant S_{20}(Corr.) was found to be 12.6 Svedberg units, and the diffusion constant D_{20}, 8.1×10^{-8} $cm.^2 sec.^{-1}$. From the value of the partial specific volume of 0.55, the molecular weight (calculated from equation 23

Page 166) becomes 8.2×10^5, and the frictional ratio f/f_0, 4.7. The corresponding axial ratio is 120.

This value of the molecular weight may be compared with the figures of 5×10^5 found by Tennent and Vilbrandt (38), and $1\text{-}2 \times 10^6$ by Schmidt et al (39).

The dimensions of the cylindrical particle then become:-

Length, $l = 2{,}538$ A

Radius $a = 10.6$ A.

In the general equation of electrophoresis of cylindrical particles (equation 21, Page 164):-

$$u = \frac{2Q}{(1+2a)F(Ka)\pi\eta}\left[\frac{K_0(Ka+Kr_i)}{(Ka+Kr_i)K_1(Ka+Kr_i)} + \ln\left(\frac{(a+r_i)}{a}\right)\right],$$

values for all the quantities are now available, and are shewn in Table II.

The charge values were obtained by plotting the valence results shewn in Table I against the ionic strength, when approximate average values are given. The values of the functions $F'(Ka)$ and $\frac{K_0(Ka+Kr_i)}{K_1(Ka+Kr_i)}$ at the various $K(a+r_i)$ values were obtained by constructing a graph from the data given by Abramson et al (13) (Graph 7/1). The value of the mean ionic radius r_i was taken as 2.24 A, this being the mean of the values for sodium and chloride obtained by Gorin (11). The value of K at 0.5° C was calculated to be $0.323 \times 10^8\sqrt{\mu}$, and the viscosity of water at this temperature was taken as 1.76×10^{-2} poise.

Table II.

μ	$\sqrt{\mu}$	$K(a+r_i)$	$F'(Ka)$	$\frac{K_0(Ka+Kr_i)}{K_1(Ka+Kr_i)}$	Averaged Q	Calculated Mobility	Observed Mobility
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

The values of the calculated, and the observed mobilities, shown in the last two columns have been plotted against the square root of the ionic strength (to which quantity they are most nearly related), to give the curves shown in Graph 7/2.

It will be observed that the correspondence between observed and calculated mobilities is very close in the lower range of ionic strength, but that increasingly large discrepancies occur as the ionic strength is raised. This is probably due in part to the greater inaccuracies of the membrane potential method in this region, and also, perhaps, partly to the increased relaxation effect in more concentrated electrolyte solutions, but it must nevertheless be concluded that the present mobility theory is only partly successful in interpreting this electrokinetic characteristic.

The partial success of this attempt to correlate the diverse colloid chemical data involved is encouraging, however, and indicates that the models used in the electrophoresis

equations do not differ fundamentally from the conditions prevailing in practice.

There is, in addition, a further conclusion from the mobility-ionic strength relationship that is of some interest. It will be observed from a comparison of the mobility-ionic strength curve (Graph 6/1) with that relating the relative viscosity to sodium chloride concentration over this range (Graph 3/7) that there is a strong qualitative resemblance between them. It would thus appear, that in accordance with the titration evidence, the viscosity changes on the addition of salts are physical effects, due largely or entirely to the change in the structure of the ionic atmosphere and decrease of zeta potential accompanying the increase of ionic strength.

The interpretation of the pH-mobility curve must be restricted at present to the qualitative picture given in Chapter VI. The similarity between the original and "back" mobility curves, when without doubt a fundamental change has occurred in the shape of the colloid micelle cannot yet be fully understood; it must be concluded that the net forces acting on the less asymetric particles produced by the change are similar to those operative beforehand. There is clearly scope for the extension of sedimentation experiments to these pH regions.

It is hoped that the present uncertainties, such as those in the derivation of the mobility equation, and the discrepancies observed when it is applied, will be resolved as the theory of electrophoretic migration is developed. In this event,

it is further hoped that the methods, the application of which has been discussed in this chapter, will prove of some value in interpreting electrophoretic phenomena in general, and help to shed additional light on the role of deoxyribonucleic acid in the life processes.

References to Chapter VII.

(1) Smoluchowski — Bull. Acad. Sci. Cracovie 1903, p 182.

(2) Helmholtz — Wied. Ann. 1879, 7, 337.

(3) Gouy — J. Phys. 1910, 9, 457.

(4) Debye and Huckel — Phys. Zeit. 1923, 24, 49, 185, 305, 575.

(5) Huckel — Phys. Zeit. 1924, 25, 204.

(6) Henry — Proc. Roy. Soc. London, 1931, A133, 106.

(7) Gorin — J. Phys. Chem. 1941, 45, 371.

(8) Abramson, Gorin and Moyer. Chem. Rev. 1939, 24, 345.

(9) Tiselius and Svensson, Trans. Farad. Soc. 1940, 36, 17.

(10) Adair and Adair — Trans. Farad. Soc. 1940, 36, 23.

(11) Gorin — J. Chem. Phys. 1939, 7, 405.

(12) Gronwall, La Mer, and Sandved — Physik. Zeit. 1928, 29, 358.

(13) Abramson, Moyer and Gorin — The Electrophoresis of Proteins, Reinhold, 1942.

(14) Harned and Owen — Physical Chemistry of Electrolytic Solutions, A.C.S. Monographs, Reinhold, 1943.

(15) Gorin, — Private Communication.

(16) Gorin and Moyer — J. Gen. Physiol. 1942, 25, 785.

(17) Svedberg, Pedersen et al. 'The Ultracentrifuge' Oxford, 1940.

(18) Lamm — Nova Acta Regiae Soc. Sci. Upsaliensis, 1937 Series 4, 10, No. 6.

(19) Perrin — J. Phys. et Radium 1936, 7, 1.

(20) Oncley — Ann. New York Acad. Sci. 1941, 41, 121.

(21) Mehl, Oncley and Simha — Science, 1940, 92, 132.

(22) Abramson — J. Gen. Physiol. 1932, 15, 575.

(23) Hammarsten — Biochem. Z. 1924, 144, 383.

(24) Greenstein — J. Biol. Chem. 1943, 150, 107.

(25) Levene and Bass — Nucleic Acids A.C.S. monograph, Reinhold, 1931.

(26) Loeb — "Proteins and the theory of Colloidal Behaviour", New York, 1922.

(27) Bjerrum — Z. Phys. Chem. 1924, 110, 656.

(28) Northrop and Kunitz — J. Gen Physiol. 1926, 9, 351.

(29) Adair and Adair — Biochem. J. 1934, 28, 199.

(30) Donnan — Z. Elektrochem 1911, 17, 572.

(31) Stadie and Hawes — J. Biol. Chem 1929, 77, 241.

(32) Linderstrom-Lang — Compt. Rend. trav. Lab. Carlberg 1926, 16, No. 16.

(33) van Rysselberghe — J. Phys. Chem. 1934, 38, 645.

(34) Mukherjee and Sarkar — J. Ind. Chem. Soc. 1947, 24, 65.

(35) Adair — Trans. Farad. Soc. 1935, 31, 130

(36) Svensson — Arkiv. Kem. Min. Geol. 1946, 22A, No. 10.

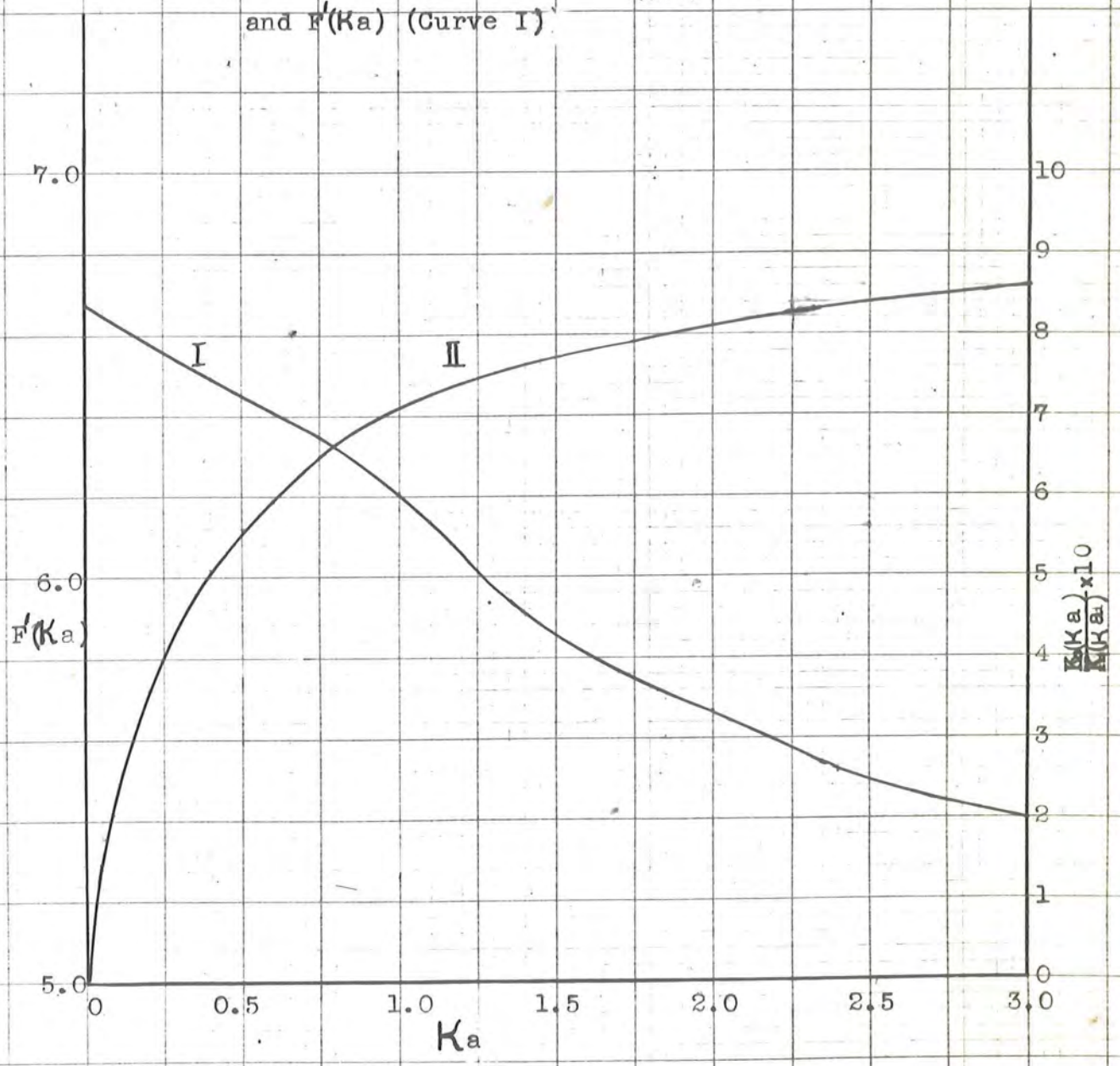
(37) Cecil and Ogston — J.C.S. in press.

(38) Tennent and Vilbrandt — J. Amer. Chem. Soc. 1943, 65, 424.

(39) Schmidt, Pickels and Levene — J. Biol. Chem. 1939, 127, 251.

GRAPH 7/1

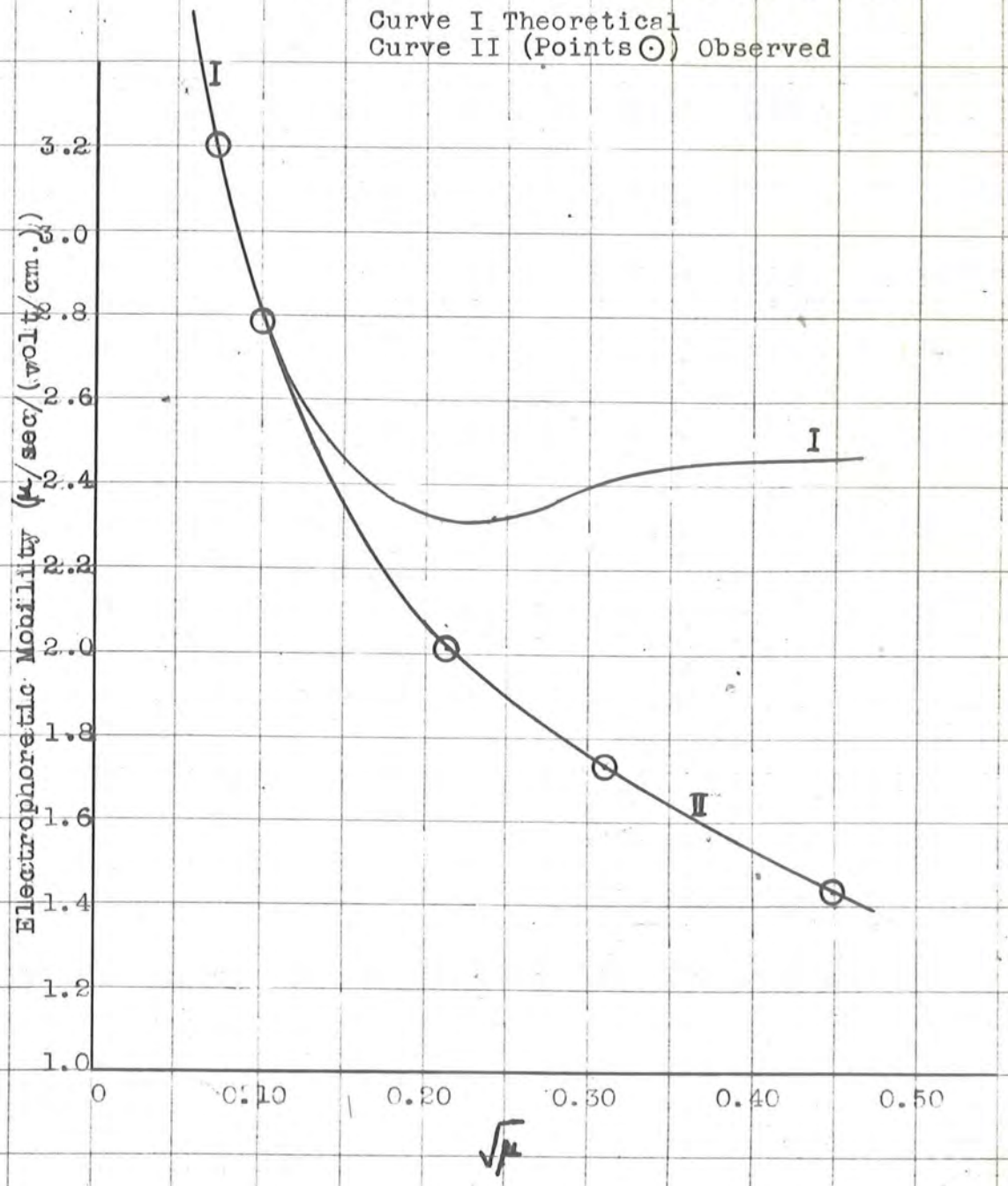
Values of the functions $\frac{K_0(Ka)}{K_1(Ka)}$ (CurveII)
and $F'(Ka)$ (Curve I)



GRAPH 7/2

Theoretical and Observed Electrophoretic Mobilities of Sodium Deoxyribonucleate.

Curve I Theoretical
Curve II (Points ⊙) Observed



Chapter VIII.

Experimental Methods.

Section (i) Viscosimetric Methods.

(ii) Preparation of Acid-and Alkali-treated samples.

(iii) Sodium Analysis.

(iv) Electrophoresis Method.

(v) Membrane Potential Methods.

Section (i).

Viscosimetric Methods.

(a) Details of the viscometers.

The viscometers, of the Frampton type, have been described and illustrated in Chapter II. The dimensions of the four in use during the present investigation are given in the table.

Viscometer No.	Radius of capillary.	Length of capillary.	"Kv"(see below)
1	0.0390 cm.	14.6 cm.	1.27 x 10
2	0.0476 cm.	13.4 cm.	2.94 x 10
3	0.0575 cm.	14.5 cm.	4.96 x 10
4	0.0965 cm.	12.0 cm.	19.5 x 10

Radius of vertical arms of all viscometers : 0.409 cm.

The distances (~0.5 cm) between the etched marks on the vertical arms of the viscometers were first measured with a travelling microscope reading to 0.01 cm, then the viscometers were calibrated by observing the times of flow of glycerol solutions of known relative viscosity (previously determined in an Ostwald viscometer calibrated against water and absolute alcohol).

From the values of the quantity $\frac{\rho \Delta t}{\log_{10}(h_1/h_2)}$ and the known relative viscosity, a viscometer constant Kv was calculated for each instrument, such that the relative viscosity η_{rel} of any solution is given by:

$$\eta_{rel} = Kv \cdot Q$$

Where Q is the particular value of the quantity $\frac{\rho \Delta t}{\log_{10}(h_1/h_2)}$.

Kv is then a measure of the ease with which a solution may pass through the viscometer. The values of Kv for the four viscometers are given in the table above.

The viscometers were cleaned after use by allowing chromic acid

solution to flow through them, then washing with water, distilled water, and finally redistilled acetone. They were dried by drawing through them a current of dust-free air.

(b) Preparation of Solutions.

All solutions were prepared, freshly each day, by dissolving the sodium deoxyribonucleate, which was dried in a vacuum over phosphorus pentoxide at 100° C, in the calculated amount of twice-distilled carbon dioxide-free water to give a concentration of 0.48%, twice the desired final value. Rapid stirring (in a covered flask) for approximately 1 hour was found necessary to give a homogeneous solution. Immediately before use in the viscometers, the nucleate solution was mixed with an equal volume of either water, salt, acid or alkaline solution of the appropriate concentration, as required, so that a final concentration of 0.24% nucleate was achieved. The solution was then transferred to the appropriate viscometer, which was clamped in a water bath thermostating at 25° C ± 0.02° and left for approximately ten minutes with the solution inside it, to attain temperature equilibrium. The viscosity determination was then carried out in the manner described in Chapter II.

(c) pH Measurement.

Hydrogen electrodes, prepared by the method of Jordan and Taylor(1) were used for all pH determinations. A calomel cell, normal with respect to potassium chloride, was used as a reference electrode, and the pH's were calculated from the observed EMF's by the formula $pH = \frac{E - 0.282}{0.058}$. On this value of 0.282 V. for the potential of the normal calomel cell, the pH of M/20 potassium hydrogen phthalate is 3.97 at 25° C.

Section (ii).

Preparation of the Acid- and Alkali-treated Samples of Sodium Deoxyribonucleate.

The four materials, the analysis and the viscosimetric behaviour of which have been recorded in Chapter III were prepared in the following manner.

An approximately 1% solution of sodium deoxyribonucleate (sample T5/1) was prepared, and treated with sufficient $\frac{N}{2}$ hydrochloric acid (or $^{N}/_{2}$ sodium hydroxide in the case of the alkali treated materials) to bring it to a pH of 3.0 (12.0 - 12.1 in the alternative case). After shaking for a few minutes the solution was neutralised (alkaline to litmus, acid to phenol phthalein) and the pH adjusted to 7.0. It was then either left to stand, or precipitated immediately.

It was found that precipitation was best effected by pouring the solution, a few mil at a time, into 98% alcohol, stirring rapidly and removing the precipitated material in each case before the addition of the next quantity of nucleate solution. By this means, rapid and almost quantitative precipitation was achieved, the recovered material being white and granular. If the whole aqueous solution was poured directly into even a very great excess of alcohol, the first precipitate was contaminated with aqueous solution, and on stirring a very fine suspension was obtained: this could not be filtered, and centrifuged only very slowly.

The collected precipitated material was washed with 80% alcohol until free from chloride, then with 90% and 100% alcohol, and finally with dry ether: drying was effected at room temperature in a vacuum over phosphorus pentoxide.

Section (iii).

Sodium Analysis.

Some samples were analysed for sodium by the colorimetric method of Hofmann and Osgood (2), whereby the solution obtained after ashing with 30% sulphuric acid and 100 vols. hydrogen peroxide is treated with zinc uranyl acetate, the mixed precipitate of 'triple salt' (sodium zinc uranyl acetate) and uranyl phosphate centrifuged off, and dissolved in ammonium thiocyanate solution, in which the triple salt is soluble and the uranyl phosphate insoluble. After centrifugation the intensity of the yellow colour, which depends on the sodium content of the original sample, was estimated by comparison with standards, using a photoelectric absorptiometer ("Spekker" Adam Hilger, Ltd. London).

The reproducibility of the results obtained by this method was low, however, and accordingly the following gravimetric method was developed, in collaboration with Dr. H. F. W. Taylor.

The weighed sample (250-500 mg) was ashed in a microKjeldahl flask with 1 ml. of concentrated nitric acid; 100 vols. hydrogen peroxide solution added in drops until the solution was colourless, and the procedure repeated if necessary. The solution was then diluted to approximately 10 ml, and placed in a bath of boiling water for 30 mins, to hydrolyse any pyrophosphate produced. Then 2 ml of 18% barium chloride solution was added, together with 1 ml of 0.880 ammonia solution saturated with ammonium carbonate. The resulting solution was then boiled for 1 minute, care being taken to avoid bumping, and to keep the solution alkaline. The precipitate of barium phosphate and barium carbonate was allowed to settle, and the solution filtered through a No. 4 Jena sintered glass filter into a 50 ml flask. the Kjeldahl flask was

then rinsed, and the precipitate on the filter washed, five times with 1 ml of hot water. The precipitate was discarded.

The solution was then boiled down to 5 ml, and made acid by the addition of two drops 30% sulphuric acid. A slight precipitate of barium sulphate formed (most of the barium precipitates as carbonate) and was removed by filtration through a similar filter (with asbestos); the filtrate was collected in a weighed platinum crucible. The flask was rinsed, and the precipitate washed five times with a few drops of hot water, and the rinsings added to the solution in the crucible. The solution was evaporated to dryness without boiling by warming on a hot plate in a current of hot filtered air. 1 ml of 30% sulphuric acid was added, and the solution evaporated over a small flame, without spitting. When cool, approximately 200 mg of solid ammonium carbonate was added, and the crucible heated to bright redness for 5 minutes. The sodium is weighed as sodium sulphate.

Trials on solutions containing sodium and phosphate in equimolecular amounts indicated that a loss of sodium occurred in the ratio 1 : 1.065 and accordingly the results were corrected by this factor.

Section (iv).

Electrophoresis Methods.

The apparatus, and its use in a typical experiment, have been described fully in Chapter V. The solutions were prepared in the same way as for the viscosity measurements, the nucleate being always dissolved first in water and brought to the correct concentration of buffer or salt by mixing with an equal volume of buffer of twice the ultimate composition. The remainder of the buffer was then mixed with an equal volume of water.

For those nucleate solutions which were treated with alkali to pH 12 before electrophoresis at a lower pH, the buffer solution must be

compensated with a corresponding amount of sodium chloride: this was done by calculating the extra sodium chloride content of the nucleate, and diluting the buffer with its own volume of dilute salt solution, of the correct concentration, instead of with water. A preliminary calculated reduction in the ionic strength of the buffer was, of course, made, so that the final ionic strength was 0.20. By this means the stationary boundaries and differing boundary velocities indicative of different salt concentrations in colloid and buffer were practically eliminated, and the need for dialysis of the solutions to ionic equilibrium obviated. The advantage of this procedure over dialysis, which is the standard method, is that the solutions could be electrophoretically examined immediately, so that the possibility of the occurrence of changes caused by prolonged acid or alkaline treatment is greatly reduced. The maintenance of low colloid concentration and high ionic strength would be expected to reduce to a minimum any effects due to the non-attainment of Donnan equilibrium, and this is borne out by the lack of boundary anomalies.

Acetate, phosphate, borate, carbonate, and veronal buffers were used, and in general contributed half only of the final ionic strength; sodium chloride was used to make up to the value of 0.20. By this means specific ion effects are minimised.

Conductivity Measurement.

In all cases the resistances of both nucleate and buffer solutions were measured in the same thermostat (0.5° C) as, and simultaneously with, the electrophoresis experiment. A valve oscillator resistance bridge (Mitcham Instruments, Ltd) at 50 cycles was used throughout; the accuracy obtainable was about 1/5 ohm at 100 ohms, using the visual null point. Except in the case of the solutions of very low ionic

strength (< 0.02) no significant difference was detectable between the resistance of the nucleate and the buffer solutions.

The electrodes of the conductivity cell used consisted of two stout platinum discs (about 1 cm apart) which were coated with platinum black. The cell was calibrated and repeatedly checked by observing the resistance of 0.100 N potassium chloride (A. R. recrystallised), dissolved in twice distilled, carbon dioxide-free water; the specific conductivity of this solution was taken as 0.00726 reciprocal ohms/cm^3. With freshly plated electrodes, the reproducibility of the null point was $\pm$ 0.05 ohm at 100 ohms resistance.

Photography.

The slides used were Ilford "Ortho" (backed) and light intensities were adjusted (by alteration of the width of the slit acting as light source) such that exposures of about 10 seconds were sufficient to give a well-defined negative. A metol-quinol developer and acid-hypo fixer were used.

Slide Measurement.

The magnification factor of the optical system was determined initially (and checked occasionally during the course of the experiments) by photographing the image of a scale fitted vertically into the stand normally occupied by the U tube. The factor had the value 0.646.

All measurements on negatives were performed with a travelling microscope, the scale of which, when magnified, could be read to 0.002 cm. The slides were clamped vertically and illuminated from one side, the accurately horizontal microscope being focussed on the other. All measurements were duplicated, and the errors in reproducibility were found generally to be less than 0.004 cm; if greater, the measurement was repeated. The cross sectional area of the U tube was determined by similar measurements.

Section (v).

Membrane Potential Methods.

(a) Preparation of the Membranes.

A solution of pyroxylin (4 gm) in a mixture of absolute ethyl alcohol (50 ml) and anhydrous ether (50 ml) was prepared, as recommended by Adair and Adair (3). Attempts to prepare membranes by coating the outside of a glass tube proved unsuccessful, but it was found that strong membranes could be obtained by two coats of the above very viscous solution on the inside of a boiling tube. By sucking a current of air through the tube, which was slowly rotated by hand, a uniform coating was obtained: by altering the time allowed for drying, membranes of differing permeabilities were prepared. When the collodion had set firmly, but before it was hard, water was poured into the tube, and introduced round the top, between the membrane and the tube. By careful manipulation with a thin glass rod, the membrane was separated from the tube, and stored in water. The membranes prepared in this manner were somewhat more porous than those described by Adair and Adair; the rate of flow of water under a pressure of 2 metres of water varied between 0.08 and 0.30 ml/minute.

The membranes were fitted with corks, which were sealed on with collodion solution. The solutions of sodium deoxyribonucleate were prepared by dissolving the dry material in the appropriate buffer or salt solution, and poured into the bag, which was then suspended in the outer solution, as shown on p176. The bag was kept sealed with a cork except during membrane potential determinations. The dialysate was replaced at intervals of one or two days, until further replacement caused no change in membrane potential.

(b) Measurement of the Membrane Potential.

The standard calomel half-cells (normal with respect to potassium chloride) were prepared in the usual way, and fitted with upturned capillary tips, the diameters of which for the two cells were approximately the same, to ensure similar liquid junctions.

When measuring the potentials, the difference between the calomels was first checked by determining the EMF between the electrodes when both cell tips were dipping into N potassium chloride solution. All EMF determinations were made with a potentiometer reading to 70 mv. and graduated in 0.02 mv., calibrated against a Weston standard cell. A Cambridge mirror galvanometer which gave a full scale deflection with ~ 2-3 mv was used to determine the null point. The probable accuracy of measurement obtainable was ~ 0.02 mv, with a somewhat higher reproducibility.

After checking the EMF difference (E cal) between the calomels, which was generally ~ 0.3 mv, the solution of potassium chloride was removed, and the tips of the cells dried with filter paper. A little liquid was forced out of the tips by gentle pressure on the rubber connecting tubes adjacent to the taps, this being absorbed by the filter paper. Release of the pressure then caused the formation of the meniscus in the capillary, about 4-5 mm. from the end of the tip.

The more negative of the two half-cells was then placed with its tip dipping into the sodium deoxyribonucleate solution within the membrane, and the more positive dipping into the dialysate. By carefully squeezing the rubber tubes, the bubbles in the capillary tips were forced out; release of the pressure then caused the formation of sharp liquid junctions about 4-5 mm from the ends.

The EMF of the cell so formed was unsteady at first, as Adair and

Adair found; after 5-10 minutes a steady reading (E_1) was obtained, and this was taken as the final value, after the method of Maclagan (4).

The two calomel cells were then removed, their tips cleaned as before, and the difference between them rechecked (E' cal). The average of the two values (which did not, in general, differ by more than 0.01 mv) was taken as the quantity to be deducted from the EMF (E_1) to give the membrane potential (E_2).

Immediately after rechecking this difference, the more positive cell was placed with its tip dipping into the nucleate solution and the more negative in the dialysate. The EMF E_3 of this cell was then measured as before, and the calomels rechecked again afterwards. The average of the calomel differences was taken as the value to be added on to the EMF to give the membrane potential, (E_4).

The results obtained in this way showed quite good agreement, as is evident from the table of results on p 198.

MEMBRANE POTENTIAL RESULTS.

Experiment No.	Buffer	μ	NaDN concn.	E_{cal}	E_1	E'_{cal}	E_2	E_{cal}	E_3	E'_{cal}	E_4	Average M.P.	Equivalent Concn.	Valence
1	NaCl	0.20	0.80%	0.15	0.80	0.21	0.62	0.21	0.45	0.19	0.64	0.63	0.0107	1090
2	NaCl	0.20	0.80%	0.02	0.58	0.03	0.55	0.20	0.33	0.20	0.53	0.54	0.00810	830
3	NaCl	0.20	0.60%	-	1.22	0.70	0.52	-	0.38	0.18	0.56	0.54	0.00810	1100
4	NaCl	0.08	0.60%	0.00	0.95	0.00	0.95	0.00	0.95	0.00	0.95	0.95	0.00647	882
5	Phosphate	0.05	0.60%	2.41	3.59	2.40	1.19	-	-	-	-	1.19	0.00511	698
6	NaCl	0.02	0.60%	0.06	2.77	0.09	2.69	0.00	2.60	0.10	2.65	2.67	0.00450	615
7	Phosphate	0.01	0.60%	0.30	4.92	0.30	4.62	0.30	4.72	0.28	5.01	4.80	0.00370	508
8	NaCl	0.005	0.60%	0.14	8.70	0.28	8.49	0.28	8.40	0.28	8.68	8.59	0.00373	509

μ = Ionic Strength, "NaDN" = Sodium Deoxyribonucleate, M.P. = Membrane potential.

All potentials in millivolts. Equivalent Concentrations in gm. equivalents/litre.

References to Chapter VIII .

(1) Jordan and Taylor — J. C. S. 1946, 994.

(2) Hofmann and Osgood — J. Biol. Chem. 1938, 124, 347.

(3) Adair and Adair — Biochem. J. 1934, 28, 199.

(4) Maclagan — Biochem. J. 1929, 23, 309.

Reprinted from the Journal of the Chemical Society, 1947.

214. *Deoxypentose Nucleic Acids. Part III. Viscosity and Streaming Birefringence of Solutions of the Sodium Salt of the Deoxypentose Nucleic Acid of Calf Thymus.*

By J. M. Creeth, J. Masson Gulland, and D. O. Jordan.

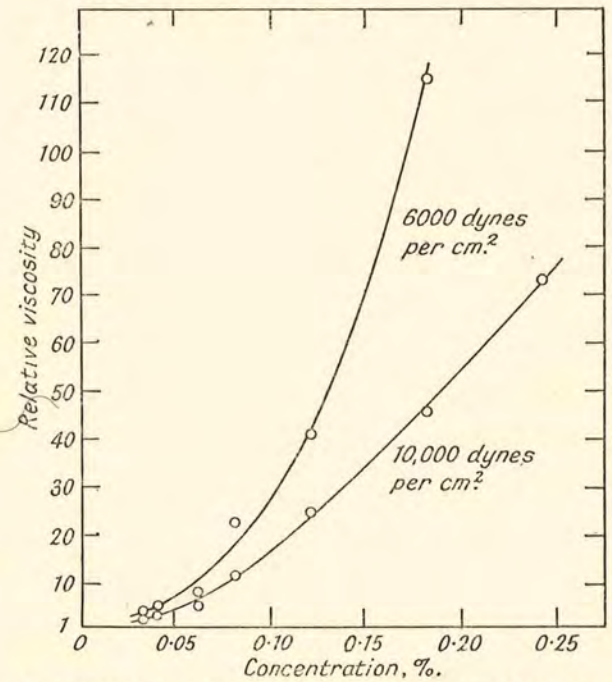
The high viscosity and marked streaming birefringence of solutions of the tetrasodium salt of deoxypentose nucleic acid of calf thymus are found to remain constant between pH 5·6 and 10·9. Outside these critical limits the viscosity falls to a very low value and the streaming birefringence disappears, but they increase again if the pH is readjusted to 7·0. The critical pH values are coincident with those at which a liberation of amino- and enolic hydroxyl groups has been observed (Gulland, Jordan, and Taylor, Part II, this vol., p. 1131) and it is considered that the two phenomena are related and are due to the fission of the hydrogen bonds postulated as linking the purine–pyrimidine hydroxyl groups and some of the amino-groups. The present data do not show whether bonding of neighbouring polynucleotide chains or of nucleotides in the same chain is involved.

The viscosities of solutions of the tetrasodium salt of deoxypentose nucleic acid of calf thymus were reduced considerably by low concentrations of neutral salt, increase of the concentration above 0·01M having relatively only a small effect on the viscosity.

The high viscosity exhibited by aqueous solutions of the sodium salt of thymus deoxypentose nucleic acid at pH 7·0 has been shown to decrease with the addition of acid and alkali (Jones and Austrian, *J. Biol. Chem.*, 1907, **3**, 1; Jones, *ibid.*, 1908, **5**, 1; Hammarsten, *Biochem. Z.*, 1924, **144**, 383; Vilbrandt and Tennent, *J. Amer. Chem. Soc.*, 1943, **65**, 1806) and with the addition of neutral salts (Greenstein and Jenrette, *J. Nat. Cancer Inst.*, 1940, **1**, 77; *Cold Spring Harbor Symp. Quant. Biol.*, 1941, **9**, 236). A mechanism involving depolymerisation has been ascribed to both these processes (Greenstein and Jenrette, *loc. cit.*; Vilbrandt and Tennent, *loc. cit.*). In view of the observations (Gulland, Jordan, and Taylor, Part II, *loc. cit.*) that

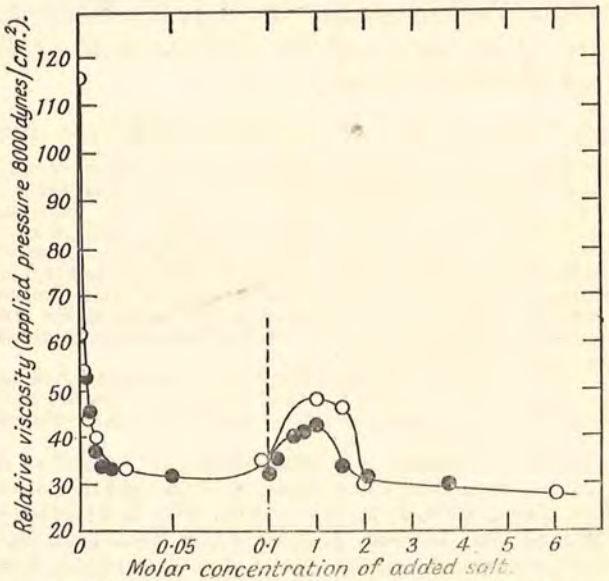
treatment with acid or alkali of solutions of the sodium salt of thymus deoxypentose nucleic acid prepared by Gulland, Jordan, and Threlfall (Part I, this vol., p. 1129) leads to the liberation

FIG. 1.



The variation of the viscosity of solutions of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus with concentration at two different pressures.

FIG. 2.



The variation of the viscosity of solutions of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus with concentration of added salt.

Sodium chloride ●; guanidine chloride ○.

of titratable groups, whereas the addition of neutral salts does not, an investigation of the viscosity of solutions of this preparation of this nucleic acid appeared desirable.

Results of this Investigation.—The viscosity of aqueous solutions of this preparation of the sodium salt of thymus deoxypentose nucleic acid increased considerably with rise of concentration (Fig. 1), and the viscosity of a 0·5% solution could not be measured in the capillary viscometers used in this investigation. At all concentrations studied the viscosity varied with the applied pressure, thus being abnormal or structural in character.

The magnitude of the relative viscosity was very much greater than that recorded for other preparations of the sodium salt of this nucleic acid. Thus, the relative viscosity of our material at pH 7·0 in 0·243% solution at 25°, measured in a capillary viscometer at 8000 dynes/cm.^{2}, was 116, whereas from measurements with the sodium salt prepared by the method of Bang (Hofmeister's " Beiträge Chem. Physiol. Path.", 1903, **4**, 331) and Hammarsten (*loc. cit.*), Vilbrandt and Tennent (*loc. cit.*) record 5·7 for a 0·3% solution at 25° measured in an Ostwald viscometer, and Greenstein and Jenrette (*loc. cit.*) give 5·53, a limiting value at high pressures, for a 0·25% solution at 25° measured in a capillary viscometer.

The addition of sodium chloride or guanidine chloride (the guanidinium ion being specified as most effective by Greenstein and Jenrette) lowered very considerably the relative viscosities of solutions of the sodium salt of thymus deoxypentose nucleic acid (Fig. 2). The viscosity fell rapidly at first as the salt concentration was increased, reaching a critical value at about 0·01M with both sodium chloride and guanidine chloride. On increasing the concentration above the critical value only comparatively small changes in viscosity occurred; a rise to a slight peak and subsequent fall were observed at approximately 1M, a result which may be compared with that observed by Needham, Kleinzeller, Miall, Dainty, Needham, and Lawrence (*Nature*, 1942, **150**, 46) for the action of neutral salts on the viscosity of solutions of myosin.

The variation of the viscosity with the pH of the solution is shown in Fig. 3; the ionic strength was maintained at 0·01 throughout. The relative viscosity remained constant as the pH was varied from 5·6 to 10·9, but outside these limits it fell rapidly, and at pH 12·08 and at pH 3·38 the viscosity of the solutions no longer varied with the applied pressure. These results are not in agreement with the data recorded by Vilbrandt and Tennent (*loc. cit.*) who observed a maximum in the relative viscosity at pH 7·0 and a gradual reduction of the relative viscosity as the pH was changed in either direction from neutrality. The results of these authors resemble those obtained by us with samples of the original sodium salt of thymus deoxypentose nucleic acid which had been treated with alkali at pH 12·5 or with acid at pH 3·5 and then precipitated by the addition of ethyl alcohol at pH 7·0 (Fig. 3). Our results with the acid- or alkali-treated material also closely resemble those obtained with a sample of the sodium salt supplied by Professor Caspersson through Professor Astbury in 1939, and prepared by the Hammarsten–Bang procedure.

The data for the streaming birefringence of solutions of the sodium salt of thymus deoxypentose nucleic acid are recorded in the table, and followed closely the changes in viscosity. In agreement with the experimental results of Greenstein and Jenrette (*J. Nat. Cancer Inst.*, 1940, **1**, 77, Table 2) and the conclusions of Snellmann and Widström (*Arkiv Kemi, Min. Geol.*, 1945, **19**, *A*, No. 31) solutions of our deoxypentose nucleic acid showed considerable streaming birefringence in the presence of a high concentration (4M) of neutral salt (see table).

Variation with pH (ionic strength maintained at 0·01 throughout) and with concentration of sodium chloride of the streaming birefringence of 0·243% solution of the sodium salt of thymus deoxypentose nucleic acid.

pH	3·7	4·0 to 4·3	4·3 to 5·0	5·0 to 10·0	10·0 to 10·7	10·9 to 11·6	12·0
Streaming birefringence (relative values on arbitrary scale)	0	1	2	3	2	1	0

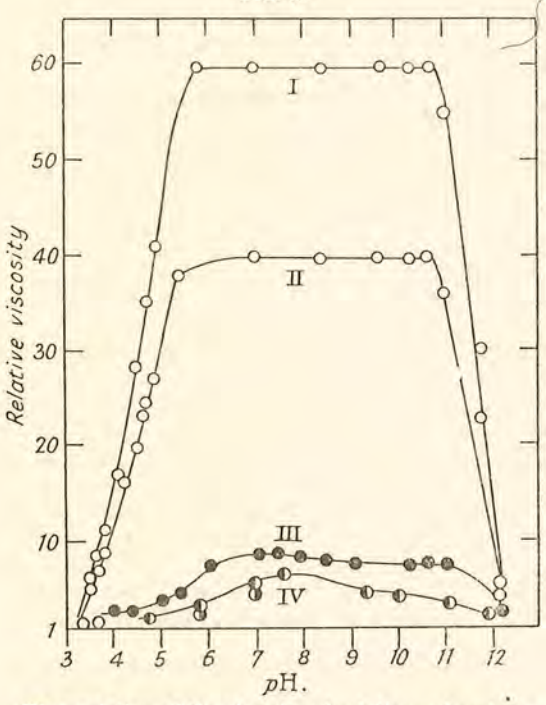
Original nucleic acid, in water at pH 6·9, 4; in 4M-sodium chloride, 2.

The action of acid and alkali in reducing the viscosity of solutions of the sodium salt of thymus deoxypentose nucleic acid has been shown by Vilbrandt and Tennent (*loc. cit.*) to be to some extent reversible if the solutions are returned to pH 7·0. We have confirmed this result, but have observed that the regain of high viscosity after acid treatment is different from that which occurs after alkaline treatment. When a 0·243% solution was left at pH 12·5 for 15 minutes and then returned to pH 7·0, the viscosity increased steadily with time (Fig. 4) and

moreover regained its structural character; after 91 hours the relative viscosity had increased to a value of the same order as that of the original acid, but the variation with applied pressure was somewhat different, the viscosity being lower at high pressures and higher at low pressures (Fig. 4). With concentrations of the sodium salt of deoxypentose nucleic acid up to 0·5% there was no appreciable increase in the viscosity at pH 7·0 after treatment at pH 3·5 for 15 minutes, but a 1·0% solution after such treatment gelled on standing for 12 hours.

When the products of alkali- or acid-treatment were precipitated at pH 7·0 by the addition of ethyl alcohol, isolated, dried, and redissolved in water, they showed only a slight increase in viscosity with lapse of time. A marked difference exists, therefore, between the behaviour of precipitated and non-precipitated material after alkali- or acid-treatment.

FIG. 3.



The variation of the viscosity of solutions of various specimens of deoxypentose nucleic acid.

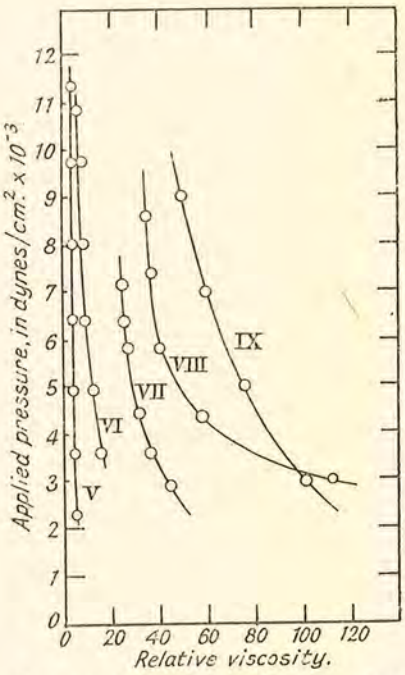
Tetrasodium salt of deoxypentose nucleic acid of calf thymus, ○ *:*

I (*applied pressure* 3000 *dynes/cm.*²), II (*applied pressure* 7000 *dynes/cm.*²).

Tetrasodium salt of deoxypentose nucleic acid after alkaline treatment, III, ●; *after acid treatment,* IV, ◐.

Tetrasodium salt of deoxypentose nucleic acid of calf thymus supplied by Professor Caspersson, IV, ◑.

FIG. 4.



Increase of viscosity of a solution of the tetrasodium salt of deoxypentose nucleic acid on standing at pH 7·0 *after alkaline treatment:* V, *after* 25 *mins.;* VI, *after* 18 *hours;* VII, *after* 42 *hours;* VIII, *after* 91 *hours;* IX, *original solution of tetrasodium salt of deoxypentose nucleic acid.*

Discussion.—The results of the viscosity measurements are interpreted qualitatively in view of the fact that the viscosity was not a function of the concentration alone over the range of concentration studied, and for such examples the theoretical treatment of viscosity data is very incomplete (Eirich, *Rep. Prog. Physics*, 1940, **7**, 329). Signer, Caspersson, and Hammarsten (*Nature*, 1938, **141**, 122) have applied one of the formulæ relating viscosity with the size and shape of the molecule, but we have not felt entitled to adopt such procedure in view of the much greater structural viscosity of solutions of our material as compared with that of the sample supplied by Professor Caspersson.

The evidence obtained by electrometric titration (Gulland, Jordan, and Taylor, *loc. cit.*) suggests that in the original nucleic acid hydrogen bonds exist between the amino- and hydroxyl groups of nucleotides, and that these bonds are broken at reactions more acid than pH 5 and

more alkaline than pH 11. The addition of acid or alkali did not lower the viscosity of solutions of deoxypentose nucleic acid until these critical pH values were reached.

The reduction in viscosity and in streaming birefringence could be explained by the rupture of hydrogen bonds between adjacent chains, producing units of lower molecular weight and greater symmetry. It is also conceivable that a rolling-up of a single polynucleotide chain could occur, following the fission of hydrogen bonds between nucleotides in that chain, thus reducing the molecular asymmetry but not the molecular weight. The present data do not reveal which of these alternatives is correct or whether both processes occur.

It is most improbable that when a solution of the sodium salt of deoxypentose nucleic acid is restored to pH 7 after acid or alkaline treatment, aggregation will produce precisely the same structure as existed in the original nucleic acid micelle, and it is likely that water molecules will play a greater part in the structure of the new micelle. Subsequent precipitation of the material at pH 7·0 by the addition of ethyl alcohol, followed by drying of the product, may thus considerably alter the structure of the micelle, and it is not surprising therefore that in solution the material isolated by precipitation behaved differently from the non-precipitated product.

The decrease in viscosity on the addition of sodium chloride cannot have been caused by a disaggregation of the type described above, since no titratable groups were produced (Gulland, Jordan, and Taylor, *loc. cit.*). At least three explanations of this decrease are possible, a disaggregation of coarse aggregates of micelles, a change in the shape of the micelle, or a change in the structure of the ion atmosphere and the hydrosphere. The data so far obtained do not permit a choice between these alternatives.

Experimental.

The determination of viscosity was made in a viscometer similar to that described by Frampton (*J. Biol. Chem.*, 1939, **129**, 233). Four viscometers were employed, having capillaries 14·6, 13·4, 14·5, and 12·0 cm. long and the following radii: 0·0390, 0·0476, 0·0575, and 0·0965 cm. respectively. The time for the liquid meniscus to fall between two marks etched on the upright tubes at a known distance apart (*ca.* 0·5 cm.) was measured with a stop watch, reading in 1/10 secs., the meniscus being followed by a travelling microscope. In the experimental results recorded in the figures, the geometric means of the initial and final hydrostatic pressures, between which the viscosity was determined, are recorded.

Streaming birefringence was determined by stirring mechanically a solution placed in a small cell on the stage of a polarising microscope.

The preparation of the materials employed has been described in Parts I and II (this vol., pp. 1129, 1131)

It is a pleasure to record our thanks to the British Empire Cancer Campaign for a maintenance grant to one of us (J. M. C.) and for defraying a part of the expenses of this investigation, and to Imperial Chemical Industries Ltd. for the loan of apparatus.

University College, Nottingham. [*Received, November 2nd*, 1946.]

Printed in Great Britain by Richard Clay and Company, Ltd.,
Bungay Suffolk.