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.

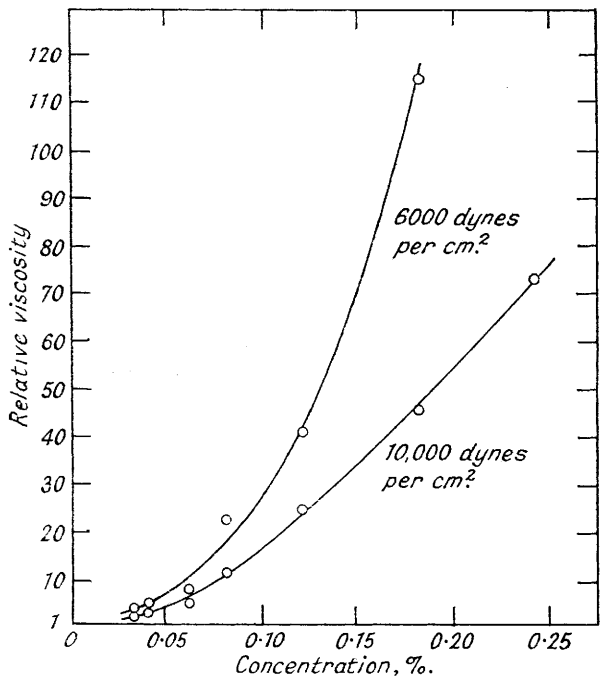
The high viscosity and marked streaming birefringence of solutions of the tetrasodium salt of deoxypentose nucleic acid of calf thymus are found to remain constant between pH 5·6 and 10·9. Outside these critical limits the viscosity falls to a very low value and the streaming birefringence disappears, 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

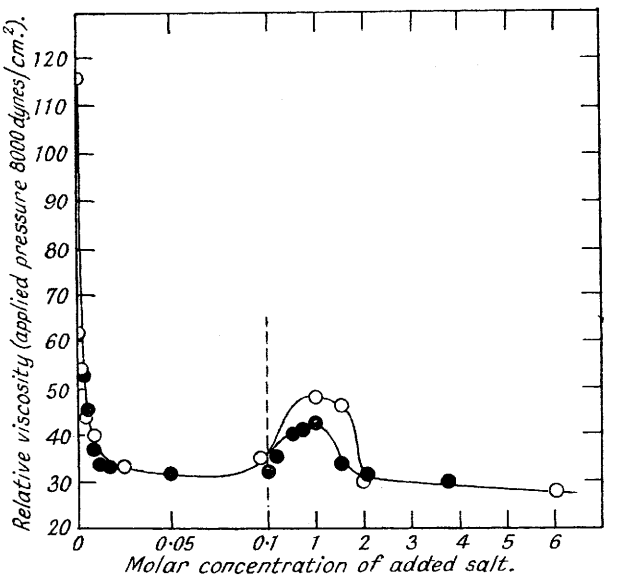
treatment with acid or alkali of solutions of the sodium salt of thymus deoxypentose nucleic acid prepared by Gulland, Jordan, and Threlfall (Part I, this vol., p. 1129) leads to the liberation

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

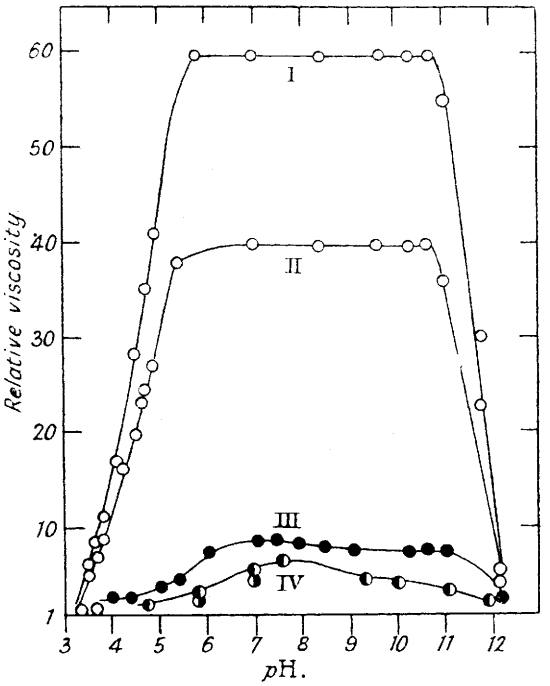
Original nucleic acid, in water at pH 6·9, 4; in 4M-sodium chloride, 2.

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.

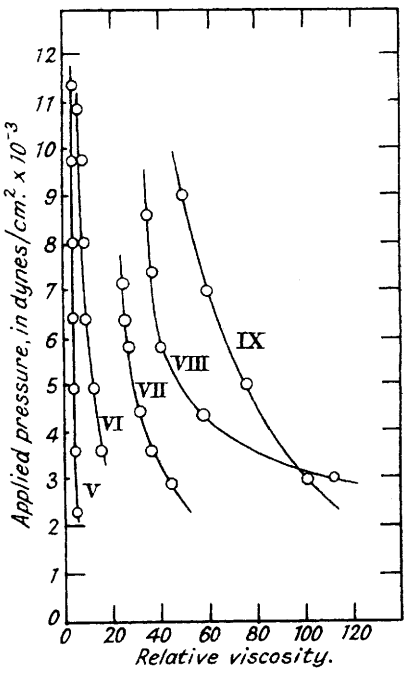
Tetrasodium salt of deoxypentose nucleic acid of calf thymus, ○ *:*

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.]