213. *Deoxypentose Nucleic Acids. Part II. Electrometric Titration of the Acidic and the Basic Groups of the Deoxypentose Nucleic Acid of Calf Thymus.*

By J. Masson Gulland, D. O. Jordan, and H. F. W. Taylor.

The acidic and the basic groups of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus and of barium thymate derived therefrom have been titrated electrometrically, employing hydrogen and glass electrodes. This polynucleotide is found to possess three amino-, two purine–pyrimidine enolic hydroxyl, four primary phosphoryl, and not more, and probably less, than 0·25 secondary phosphoryl dissociations for every four atoms of phosphorus. These data are consistent with a chain structure for this acid in which branching, if it occurs, is infrequent as compared with yeast ribonucleic acid. The main internucleotide bond is an ester linkage. For every four atoms of phosphorus it is found that there are 1·0 guanine, 1·0 thymine, 1·0 to 1·2 cytosine, and 1·0 to 0·8 adenine radicals.

The initial dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus obtained by titrating from pH 6·9 with acid and alkali are abnormal, being displaced from the back-titration curves. This discrepancy between the forward- and back-titration curves persists in high concentrations of neutral salt. It is concluded that the purine–pyrimidine hydroxyl groups and some of the amino-groups are blocked, most probably by a hydrogen bond between these groups. The significance of this linkage in the macromolecular structure of the tetrasodium salt of the deoxypentose nucleic acid is discussed.

The conflicting results obtained previously from investigations of the acid–base properties of thymus deoxypentose nucleic acid (Steudel, *Z. physiol. Chem.*, 1912, **77**, 497; Feulgen, *ibid.*, 1919, **104**, 189; Levene and Simms, *J. Biol. Chem.*, 1925, **65**, 519; 1926, **70**, 327; Makino, *Z. physiol. Chem.*, 1935, **232**, 229; 1935, **236**, 201; Bredereck, Köthnig, and Lehmann, *Ber.*, 1938, **71**, 2613; Bredereck and Köthnig, *ibid.*, 1939, **72**, 121; Ahlström, Euler, Fischer, Hahn, and Högberg, *Arkiv Kemi, Min. Geol.*, 1945, **20**, *A*, 1) may be ascribed to the different degrees of degradation of the samples studied (Schmidt, Pickels, and Levene, *J. Biol. Chem.*, 1939, **127**, 251; Cohen, *ibid.*, 1942, **146**, 471; Tennent and Vilbrandt, *J. Amer. Chem. Soc.*, 1943, **65**, 424; Gulland, Barker, and Jordan, *Ann. Rev. Biochem.*, 1945, **17**, 175). In all probability the least degraded specimens which have hitherto been examined were those prepared by the method of Bang (Hofmeister's " Beiträge chem. Physiol. Path.", 1903, **4**, 331) and studied conductimetrically by Hammarsten (*Biochem. Z.*, 1924, **144**, 383) and electrometrically by Jorpes (*Biochem. J.*, 1934, **28**, 2102) and Stenhagen and Teorell (*Trans. Faraday Soc.*, 1939, **35**, 743). They were found to possess four acid-dissociating groups per hypothetical tetranucleotide, having the very approximate pK_a' values of 2·4, 3·7, 4·3, and 5·2. The Hammarsten–Bang method of isolation, however, gave a product which on analysis was found to be very deficient in nitrogen and phosphorus (N, 11·97; P, 7·09%; Hammarsten, *loc. cit.*) when compared with the theoretical for the tetrasodium salt (N, 15·85; P, 9·37%). Furthermore, Hammarsten studied the free acid obtained from the sodium salt by the action of hydrochloric acid; as will be shown in this paper this treatment causes an irreversible change in that the free acid isolated from solutions more acid than pH 3·5 does not show the same acid–base properties as the sodium salt isolated at pH 7·0.

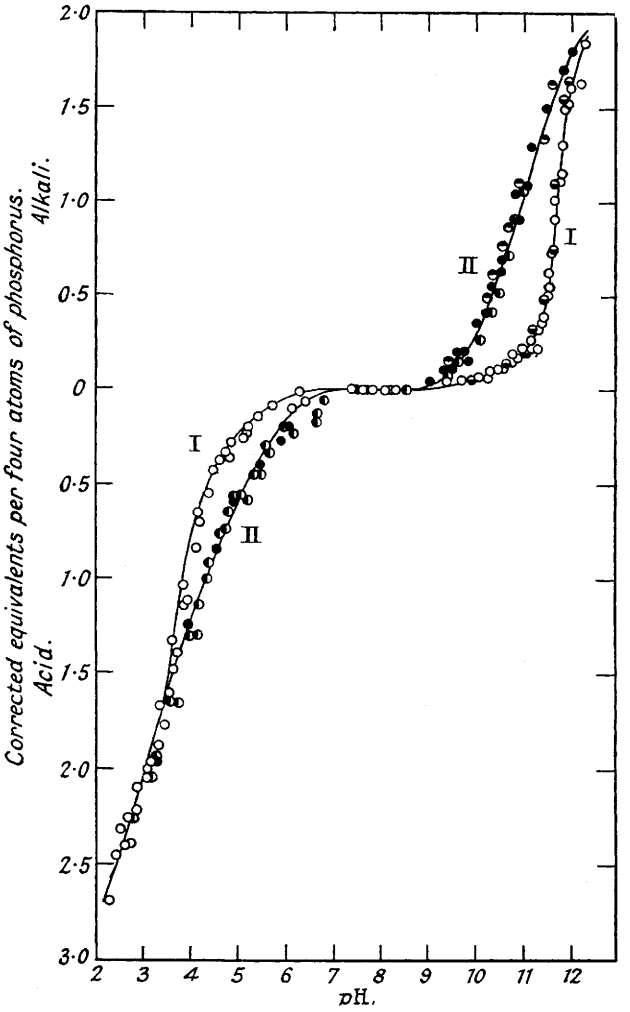
The sodium salt of calf thymus deoxypentose nucleic acid which has been studied in this investigation was isolated by a mild method (Gulland, Jordan, and Threlfall, Part I, this vol., p. 1129), throughout which the solution employed did not vary significantly from pH 7·0.* The solid was fibrous and dissolved in water to give a faintly opalescent solution, having a pH of 6·90, which exhibited marked structural viscosity and streaming birefringence (Creeth, Gulland, and Jordan, Part III, this vol., p. 1141). A second specimen, prepared by the Hammarsten–Bang procedure and supplied by Professor Caspersson through Professor Astbury in 1939, has also been studied. It was found to contain a small amount of protein which was removed by the method of Sevag, Lackman, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425), and is believed to be identical with that studied with ultracentrifuge and viscosity methods by Signer, Caspersson, and Hammarsten (*Nature*, 1938, **141**, 122) and with *X*-ray methods by Astbury and Bell (*ibid.*, p. 747).

* It is necessary to correct a point in the paper of Tennent and Vilbrandt (*J. Amer. Chem. Soc.*, 1943, **65**, 424). The sample of " Thymonucleic acid TNA2, prepared by Gulland ", referred to by these authors, was a purchased commercial sample, and the " barium thymate BT1, prepared by Gulland " was made from it by the usual method. These were given to Professor Astbury in 1939 with a warning that their purity and homogeneity was open to doubt; they were not intended for the type of investigation to which they have been put by Tennent and Vilbrandt, and in our view results obtained with them are of no value in connection with nucleic acid structure.

Results of the Present Investigation.—All electrometric titration curves have been corrected at the extremes of pH for the titration of the water by the method of Jordan and Taylor (*J.*, 1946, 994).

(i) *Titration of the sodium salt of thymus deoxypentose nucleic acid.* The titration curve of this sample is shown in Fig. 1, curve I. It will be seen that on the addition of acid or alkali to

FIG. 1.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus :

I. *Titration with acid or alkali from pH* 6·9, ○, ◒. *The smooth curve drawn through these points is calculated for* 1 *equiv. each of* pK_a' *values* 2·6, 3·5, 5·2, 10·4 and 11·4.

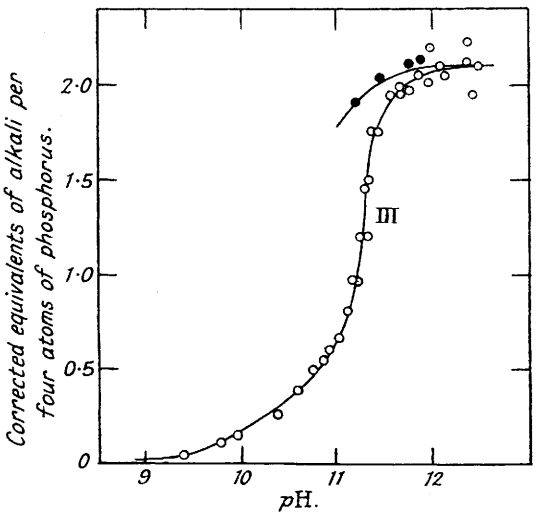
II. *Back titration with acid from pH* 12·0, ●, ◓ *and with alkali from pH* 2·5, ◐.

Points marked ◒ *and* ◓ *obtained with " Alki " glass electrode (Cambridge Instrument Co., Ltd.) ; all other points obtained with hydrogen electrode.*

the solution in water no groups are titrated at first between pH 5·0 and 11·0, but that outside these limits there occurs a rapid liberation of groups titrating in the ranges pH 2·0 to 6·0 and pH 9·0 to 12·0. On back titration either with acid from pH 12·0 or with alkali from pH 2·5, a curve (II) is obtained which is different from that representing the initial titration, and it is significant that the same curve (II) is obtained whether the back titration is with alkali from pH 2·5 or with acid from pH 12·0. This complete identity of the back-titration curves suggests that acid and alkali have an identical effect in liberating both sets of groups.

The back-titration curve exhibits a well-defined point of inflection in the neutral region, and shows incipient points of inflection in the regions of pH 12·0 and pH 2·0, corresponding respectively to approximately 2·0 equivalents of alkali and 3·0 equivalents of acid for each four atoms of phosphorus. There is some difficulty in interpreting electrometric titration

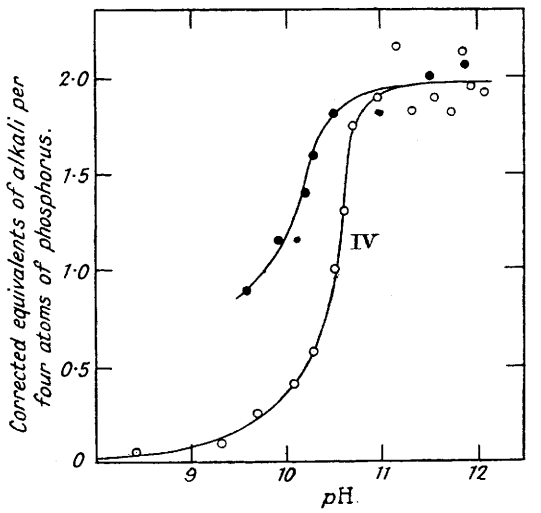
FIG. 2.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus in M*-potassium chloride.*

Titration with alkali from pH 6·9, ○ ; *back titration with acid from pH* 12·5, ●.

FIG. 3.



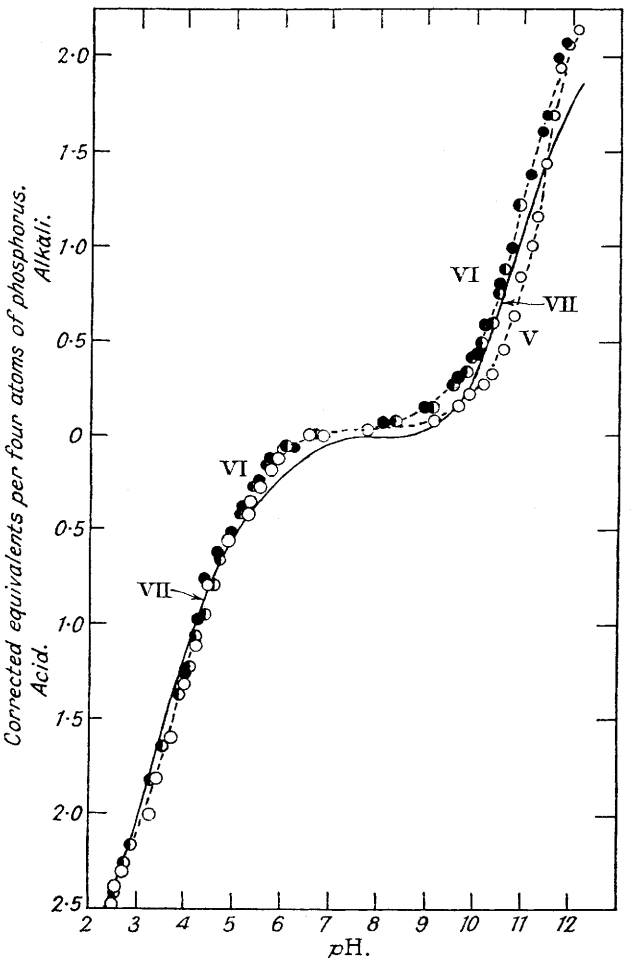
The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus in 2·5M*-guanidine sulphate.*

Titration with alkali from pH 6·9, ○ ; *back titration with acid from pH* 12·0, ●.

data above pH 11·0 and below pH 3·0 owing to the dependence of the water correction on the ionic strength (Jordan and Taylor, *loc. cit.*), which, for a polybasic substance such as nucleic acid, cannot be estimated with certainty. In obtaining the data given in Fig. 1, the assumption has been made that the acidic and basic groups contribute independently to the ionic strength. This approximation is justified by the fact that no appreciable proportion of the phosphorus

atoms carry more than one dissociating group (see below). It appeared desirable, however, to have additional confirmation of the number of groups titrating in the alkaline region, and titration of the sodium salt of thymus deoxypentose nucleic acid was therefore carried out in M-potassium chloride. The presence of the potassium chloride has the effect of masking all other contributions to the ionic strength (Cohn, Green, and Blanchard, *J. Amer. Chem. Soc.*, 1937, **59**, 509), and the water correction is obtained from a titration of M-potassium chloride. The results of this titration, which are given in Fig. 2, curve III, show conclusively that the

FIG. 4.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus after alkaline treatment followed by precipitation with alcohol at pH 7:

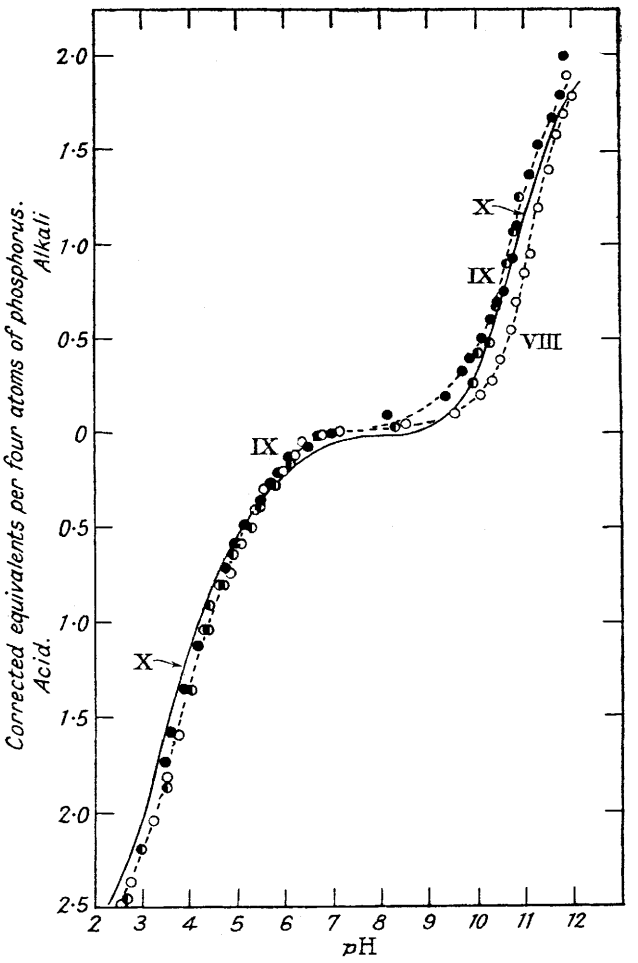
V. *Titration with alkali from pH* 6·7, ○.
VI. *Titration with acid from pH* 6·7, ○; *back titration with alkali from pH* 2·5, ◐; *back titration from pH* 12·0, ●.
VII (*full curve*). *Mean titration curve of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus from Fig.* 1.

number of groups dissociating in the range pH 8·0 to 12·0 is 2·05. Owing to the ease with which precipitation of the deoxypentose nucleic acid occurs in the presence of M-potassium chloride when acid is added, a complete back titration below pH 11·2 was not practicable; the data which have been obtained, however, are sufficient to indicate that the discrepancy between the forward- and back-titration curves described above persists in the presence of M-potassium chloride. Very similar results were obtained by titration in 2·53M-guanidine sulphate ($[C(NH_2)_3]_2SO_4$; Fig. 3, curve IV). Owing, however, to the existence of an unsteady liquid-

junction potential between this solution and the saturated potassium chloride bridge, the pH values were not very reproducible, especially in strongly alkaline solutions.

(ii) *Titration of the sodium salt of thymus deoxypentose nucleic acid precipitated with alcohol at pH* 7·0 *after treatment with alkali at pH* 12·0, *or with acid at pH* 3·0. The titration curves of an alkali-treated sample are shown in Fig. 4, curves V and VI; similar results were obtained with two other samples. The results of titration on the alkaline side of neutrality resemble those obtained with the original substance, in that a shift in the dissociation curve is observed

FIG. 5.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus after acid treatment followed by precipitation with alcohol at pH 7 :

VIII. *Titration with alkali from pH* 7·1, ○.
IX. *Titration with acid from pH* 7·1, ○ ; *back titration from pH* 2·3, ◐ ; *back titration from pH* 12·0, ●.
X (*full curve*). *Mean titration curve of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus from Fig.* 1.

on back titration, although to a somewhat smaller extent. No such effect, on the other hand, was observed on the acid side. The back-titration curves from pH 12·0 or pH 2·5 are identical, and are very similar to the curve obtained with the original substance.

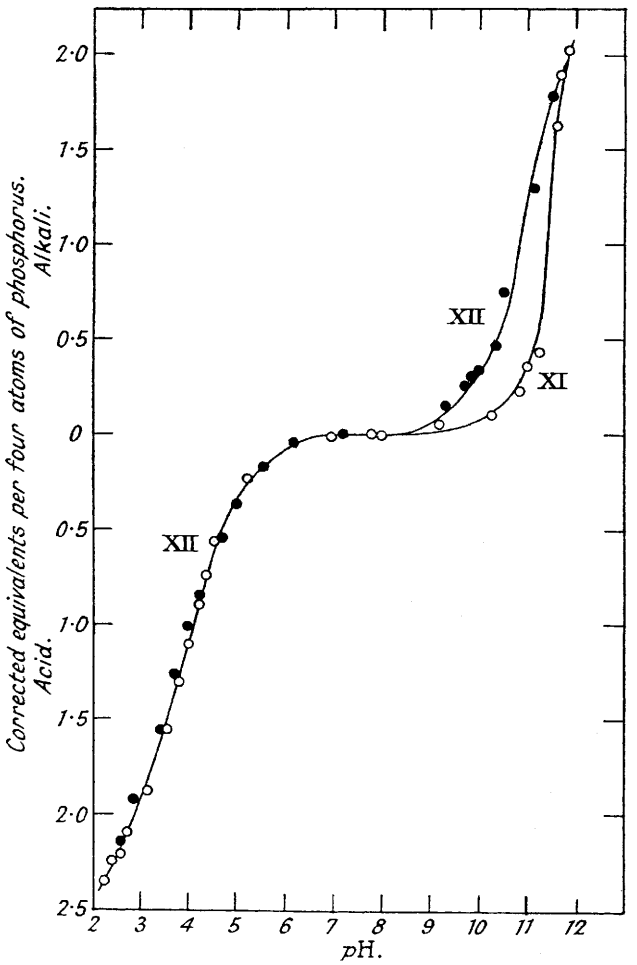
The results obtained with an acid-treated specimen (Fig. 5, curves VIII and IX) are almost identical with those described above for the alkali-treated material.

(iii) *Titration of the sodium salt of thymus deoxypentose nucleic acid supplied by Professor Caspersson.* The titration curves of this sample are shown in Fig. 6, curves XI and XII, and

are intermediate between those of the alkali- and acid-treated materials prepared by us and those of our original material. Viscosity studies (Creeth, Gulland, and Jordan, *loc. cit.*) support the view that, compared with the acid prepared by us, the sample of Caspersson exhibits different, probably less, hydrogen bonding (see below) and lower viscosity.

(iv) *Titration of the barium salt of thymic acid.* On treating thymus deoxypentose nucleic acid with dilute sulphuric acid at 80°, quantitative removal of guanine and adenine takes place (Feulgen, *Z. physiol. Chem.*, 1918, **101**, 296; Feulgen and Landmann, *ibid.*, **102**, 262; Bredereck

FIG. 6.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus supplied by Professor Caspersson :

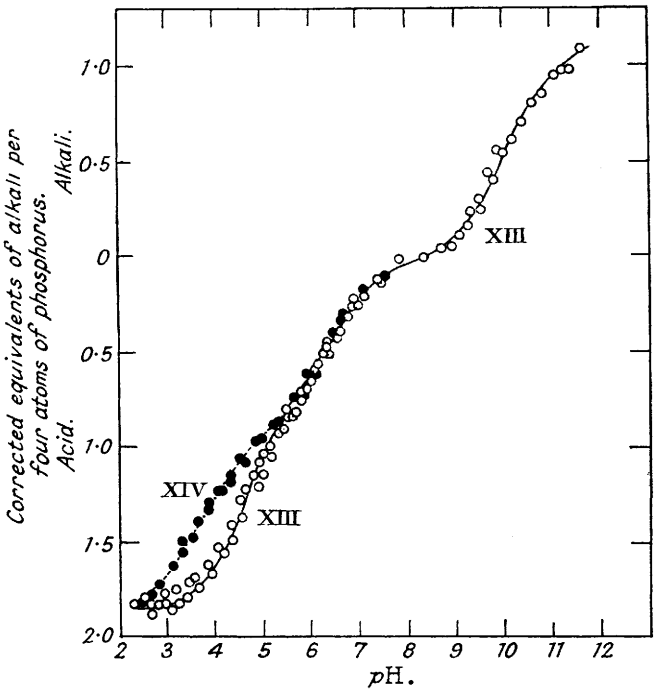
XI. *Titration with alkali from pH* 7·8, ○.
XII. *Titration with acid from pH* 7·8, ○ ; *back titration from pH* 11·8, ●.

and Müller, *Ber.*, 1939, **72**, 115); the resulting thymic acid is isolated as its barium salt. The empirical formula weight of this substance is defined, for convenience, as the amount containing 4 g.-atoms of phosphorus, and the values obtained from the phosphorus contents of the two preparations studied were 1310 and 1315. The electrometric titration curve calculated on the basis of these formula weights is shown in Fig. 7, curve XIII, well-marked points of inflection being observed at pH 2·5, 8·0 and 11·5 after the neutralisation of 1·85 and 1·0 equivalents respectively. A titration has also been carried out in the presence of formaldehyde in order to ascertain what proportion of the more acidic dissociation represents that of an amino-group (curve XIV).

Discussion.—(i) *The nature of the acidic and the basic groups of deoxypentose nucleic acid.* The problem of the initial structure of deoxypentose nucleic acid before alkali or acid treatment will be considered in section (ii) of this discussion, and in this section the back-titration curve (Fig. 1, curve II) only will be treated.

Owing to the fact that the deoxyribonucleotides have not yet been isolated in quantities sufficient to permit an investigation by electrometric methods, it is necessary to refer to dissociation-constant data of the ribonucleotides in order to interpret this curve. This procedure is to some extent justified by the fact that the pK_a' values of the amino-groups of adenylic

FIG. 7.



The dissociation curves of the barium salt of thymic acid :

XIII. *Titrations with acid and alkali from approximately pH* 7, ○. *The smooth curve drawn through these points is calculated for* 1·1 *equivs. of* pK_a' 4·6, 0·75 *equiv. of* pK_a' 6·5, *and* 1·0 *equiv. of* pK_a' 10·0.

XIV. *Titration in* 1·875% *formaldehyde solution,* ●.

(*The zero of equivalents is fixed arbitrarily at pH* 8·4, *the titrations being carried out on two samples of slightly different barium content.*)

and cytidylic acids do not differ very greatly from those of adenine and cytosine respectively, as shown by the following data :

Adenine	4·15	Adenylic acid	3·70 [1]
Cytosine	4·60 [1]	Cytidylic acid	4·24 [1]

[1] Data of Levene and Simms (*loc. cit.*).

It is not expected, therefore, that replacement of *d*-ribose by deoxypentose would give rise to any considerable change in the pK_a' values.

There has been some confusion in the literature between the pK_a' values assigned to the amino- and the primary phosphoryl dissociations of nucleic acids. Levene and Simms (*loc. cit.*) considered the groups dissociating in the range pH 2·0 to 6·0 to be the amino-groups, whereas Hammarsten (*loc. cit.*) and Fletcher, Gulland, and Jordan (*J.*, 1944, **33**) considered them to be the primary phosphoryl dissociations. Consideration of the pK_a' values for the

nucleosides and sugar phosphates (given below) in the light of the modern theory of zwitterions points conclusively to the former view being correct.*

Adenosine ..... 3·45	(Levene and Simms, *loc. cit.*).
Sugar phosphates... pK_{a_1}', 0·8 to 1·1; pK_{a_2}', 6·0 to 6·5	(Kumler and Eiler, *J. Amer. Chem. Soc.*, 1943, **65**, 2355).
Adenylic acid ..... pK_{a_1}', 0·89; pK_{a_2}', 3·70; pK_{a_3}', 6·01	(Levene and Simms, *loc. cit.*).

The pK_{a_1}' value of adenylic acid is clearly that of a primary phosphoryl dissociation, the pK_{a_2}' an amino-dissociation, and the pK_{a_3}' a secondary phosphoryl dissociation. At the isoelectric point, therefore, nucleic acids will exist almost entirely in the zwitterionic form.

The dissociations which will be titrated in the range pH 2·5 to 8·0 are therefore those of the amino- and the secondary phosphoryl groups. Examination of the lower portion of the back-titration curve (Fig. 1, curve II) shows that groups of both types are present, although the amount of secondary phosphoryl dissociation for every four atoms of phosphorus is relatively very small (see below). The curve is in approximate agreement with a theoretical curve constructed for 1·0 equivalent each, for every four atoms of phosphorus, of the amino-dissociations of guanylic, adenylic, and cytidylic acids, the pK_a' values of which are 2·3, 3·7, and 4·24 respectively (Levene and Simms, *loc. cit.*; Fletcher, Gulland, and Jordan, *loc. cit.*). It appears, however, that the pK_a' value of the amino-group of cytidylic acid (*viz.*, 4·24) which has been assumed for the amino-group of cytosine deoxypentose nucleotide is low, and much better agreement with the experimental curve is obtained by employing the pK_a' values of 2·5, 3·5, and 5·2 for constructing the theoretical curve; the curve shown in Fig. 1 is calculated on this basis.

The titration curve in the range pH 5·5 to 7·5 indicates the presence of a small amount of a group having a pK_a' value of 6·0 to 6·5, which is considered to be a secondary phosphoryl group. The determination of the exact quantity of this dissociation is dependent upon a precise knowledge of its pK_a' value and of the amounts of the amino-dissociations and their pK_a' values, but using 6·5 for the pK_a' value of the secondary phosphoryl dissociation, which is that observed for thymic acid, and assuming that there is no overlap of the amino- and the secondary phosphoryl dissociations above pH 6·5 (*i.e.*, no amino-dissociation greater than pK_a' 4·5), the amount of secondary phosphoryl dissociation is 0·25 equivalent for every four atoms of phosphorus. This represents the maximum amount of this group which can be present. Since, however, the pK_a' value of the weakest amino-group is of the order of 5·2, overlapping of the dissociations above pH 6·5 must occur, and thus the amount of the secondary phosphoryl dissociation present will be less than the maximum value.

The analysis of the sodium salt of deoxypentose nucleic acid shows that there are four sodium atoms for every four atoms of phosphorus, and in view of the fact that the amount of secondary phosphoryl dissociation is small, the four atoms of sodium must be combined largely or entirely with four primary phosphoryl dissociations. The deoxypentose nucleic acid of calf thymus differs in this respect markedly from the ribonucleic acids of yeast (Fletcher, Gulland, and Jordan, *loc. cit.*) and of *Calliphora erythrocephela* (Khouvine and Grégoire, *Bull. Soc. Chim. biol.*, 1944, **16**, 421), both of which show 1·0 equivalent of secondary phosphoryl dissociation (on correction for the phosphorus analysis) for every four atoms of phosphorus, and it cannot therefore possess the branched chain structure postulated for yeast ribonucleic acid by Fletcher, Gulland, and Jordan (*loc. cit.*). The data recorded are, however, consistent with the view that the thymus deoxypentose nucleic acid of calf thymus has a long, unbranched chain structure (Signer, Caspersson, and Hammersten, *loc. cit.*; Astbury and Bell, *loc. cit.*).

The groups titrating in the range pH 8·0 to 12·0 are considered to be the purine–pyrimidine hydroxyl groups of thymine and guanine deoxypentosides, and the upper part of the back-titration curve is in agreement with a theoretical curve constructed for 1·0 equivalent each of pK_a' values 10·4 and 11·4. The corresponding pK_a' value for thymine is 9·94 (Levene, Bass, and Simms, *J. Biol. Chem.*, 1926, **70**, 229; and confirmed by us) and for guanylic acid, 9·36 (Levene and Simms, *loc. cit.*), which, although of the same order of magnitude as the upper dissociations of deoxypentose nucleic acid, are nevertheless appreciably lower. The same effect is seen to a lesser extent in yeast ribonucleic acid which shows 2·0 dissociations of pK_a' 10·2 as compared with 9·36 for guanylic acid and 9·43 for uridylic acid (Levene and Simms, *loc. cit.*; Fletcher, Gulland, and Jordan, *loc. cit.*). The reason for this discrepancy is not clear,

* Acceptance of this view does not alter the main conclusions of Fletcher, Gulland, and Jordan about the structure of yeast ribonucleic acid, except that the triply-bound phosphoryl group is not necessarily that of uridylic acid.

especially in view of the better agreement which exists between the pK_a' values for the amino-dissociations of the ribonucleotides and of a yeast ribonucleic acid. It may, however, be related to the degree of polymerisation of the nucleic acid since it is not observed in thymic acid, which is considered to have a low molecular weight (see below).

The data given in Fig. 2, curve III, show that, other than the purine–pyrimidine hydroxyl groups, there are no dissociating groups titrating with alkali which have a pK_a' value in water less than at least 13·5. This confirms the absence of free sugar hydroxyl groups in this sample, since the pK_a' values of the primary hydroxyl dissociations of many sugars are in the region of 12·5 (Hirsch and Schlags, *Z. physikal. Chem.*, 1929, **141**, *A*, 387; Stearn, *J. Physical Chem.*, 1931, **35**, 2226; Urban and Shaffer, *J. Biol. Chem.*, 1932, **94**, 697; Urban and Williams, *ibid.*, 1933, **100**, 237), and suggests that no other sugar than a deoxypentose is present in any appreciable quantity in this sample of deoxypentose nucleic acid of thymus. Furthermore, taken in conjunction with the presence of one primary phosphoryl dissociation for every atom of phosphorus, the guanine, adenine, and cytosine dissociations and the guanine and thymine hydroxyl dissociations, this fact supports the view that the internucleotide bond is an ester linkage between the phosphoryl groups and the two hydroxyl groups of the sugar which are not involved in the glycosidic ring structure. Other types of linking may, however, exist in the nucleic acid, but the sensitivity of the titration method precludes the occurrence of such other linkages to a greater extent than one for every ten to twenty nucleotides.

The titration of thymic acid, taken in conjunction with the preceding data for deoxypentose nucleic acid, supplies information concerning the proportions of the four bases guanine, adenine, cytosine, and thymine present in this sample of nucleic acid. The titration curve for thymic acid (Fig. 7) shows 1·0 dissociation per four atoms of phosphorus in the pH range 8·0 to 12·0; this group can only be the enolic hydroxyl group of thymine. The 2·0 dissociations therefore observed in this pH range for deoxypentose nucleic acid (see above) must indicate the presence of one molecule each of thymine and guanine for every four atoms of phosphorus. It is not possible to determine with certainty the relative proportions of cytosine and adenine from the titration data of the sodium salt of deoxypentose nucleic acid, although the total amount of these groups is approximately 2·0. Thymic acid, however, possesses for every four atoms of phosphorus 1·0 to 1·2 dissociations of pK_a' 4·5 which must be the amino-dissociation of cytosine, and thus it is probable that the ratio of cytosine to adenine in the deoxypentose nucleic acid is as 1·0—1·2 is to 1·0—0·8.

The presence in barium thymate of 0·75 equivalent of a secondary phosphoryl dissociation for every four atoms of phosphorus suggests, on the basis of the straight-chain structure for deoxypentose nucleic acid, that the average number of nucleotides per molecule of thymic acid is approximately 5, and thus that the average molecular weight of the free acid is of the order of 1200.

(ii) *The macromolecular structure of deoxypentose nucleic acid.* As is shown in Fig. 1, the amino- and the enolic hydroxyl groups of thymus deoxypentose nucleic acid are partly or completely blocked until the material has been treated with acid or alkali; an irreversible change then takes place with the accompanying liberation of titratable groups. The release of the groups on treatment with alkali takes place sharply in the neighbourhood of pH 11·5, but less sharply in the range pH 3·5 to 4·5 on treatment with acid. In both cases equilibrium is established almost instantaneously, and the liberation of groups is accompanied by a marked fall in the viscosity and a disappearance of streaming birefringence (Hammarsten, *loc. cit.*; Vilbrandt and Tennent, *J. Amer. Chem. Soc.*, 1943, **65**, 1806; Creeth, Gulland, and Jordan, *loc. cit.*).

The decrease in viscosity brought about by the addition of acid or alkali was considered by Vilbrandt and Tennent (*loc. cit.*) to be caused by a depolymerisation which was slowly reversed when the solution was returned to neutrality. Our results show that such a depolymerisation cannot involve the rupture of the internucleotide ester linkages since no increase of secondary phosphoryl dissociation is observed in the back-titration curve. The complete identity of the back-titration curves from pH 2·5 and pH 12·0 strongly suggests that acid and alkali have the same effect in liberating the amino- and the hydroxyl groups. Two possibilities may be considered to explain this behaviour. It could be caused by easily hydrolysed radicals, hitherto unidentified in the breakdown products of the nucleic acid, which either substitute in the amino- and the hydroxyl groups separately or form a bridge between them. There are, however, certain limitations in the type of radical which could be involved; first, it could not contain groups which are titrated in the pH range examined in this investigation, since the back-titration curve shows no liberation of such groups and is moreover almost identical with the titration curve of the acid or alkali treated samples (Figs. 4 and 5), and secondly, the stability

to acid and alkali of the links involving the amino- and the hydroxyl groups would have to be very similar. In our opinion these restrictions make the preceding explanation of the observed behaviour improbable, and a simpler and preferable explanation is that in which the amino- and the hydroxyl groups are linked by hydrogen bonds. Bonding of this type has frequently been suggested as being important in protein structure, and our observations on the behaviour of deoxypentose nucleic acid resemble in some respects those recorded for egg albumen (Cannan, Kibrick, and Palmer, *Ann. N.Y. Acad. Sci.*, 1941, **41**, 243; Crammer and Neuberger, *Biochem. J.*, 1943, **37**, 302), which have been interpreted by postulating a hydrogen bond between a phenolic hydroxyl group and a carboxylate ion. Although it is undesirable at the present stage to speculate too far as to the macromolecular structure of deoxypentose nucleic acid, a hydrogen bond between an amino-group and either the $-NH-$ group or the enolic $-C(OH)-$ group of an adjacent guanine or thymine radical could explain satisfactorily our experimental results. The large number of such bonds which are possible, the maximum number being two for every four atoms of phosphorus, would give a degree of stability to the untreated deoxypentose nucleic acid, and in order to degrade the nucleic acid it might be necessary to break many of the hydrogen bonds simultaneously. Such a process would lead to an abnormal titration curve of the type shown in Fig. 1. It is not possible on the basis of these data to decide whether the hydrogen bonds unite nucleotides in the same, or in different chains; this aspect is considered in the light of viscosity and streaming birefringence by Creeth, Gulland, and Jordan (*loc. cit.*).

The increase in viscosity observed at approximately pH 7 when a solution of the nucleic acid was adjusted to that value after treatment with acid or alkali (Vilbrandt and Tennent, *loc. cit.*; Creeth, Gulland, and Jordan, *loc. cit.*) does not involve the blocking of the amino- and the enolic hydroxyl groups, since the titration curves obtained were identical with the back-titration curve shown in Fig. 1 whether the solution was titrated immediately or was allowed to remain at approximately pH 7 for 96 hours in the absence of atmospheric oxygen. During this period the viscosity had risen to a value of the same order as that observed with the original acid (Creeth, Gulland, and Jordan, *loc. cit.*).

A different behaviour was observed in samples which had been precipitated by alcohol at pH 7 after acid or alkaline treatment. Some blocking of the enolic hydroxyl groups occurred (Figs. 4 and 5), but to a much smaller extent than that found with the original material, and the properties of the precipitated material appeared to be independent of the viscosity changes, since very similar titration curves were obtained whether the product was precipitated immediately or after 96 hours. Precipitation would thus seem to be the important factor. There is no evidence from titration data that precipitation effects blocking of the amino-groups.

Greenstein and Jenrette (*J. Nat. Cancer. Inst.*, 1940, **1**, 77; *Cold Spring Harbor Symp. Quant. Biol.*, 1941, **9**, 236) have postulated on the evidence of viscosity measurements that reversible depolymerisation of thymus deoxypentose nucleic acid takes place on the addition of neutral salts. Titration of the sodium salt of thymus deoxypentose nucleic acid in M-potassium chloride (Fig. 2) and in 2·53 molar guanidine sulphate (Fig. 3) showed that the changes in viscosity which occur bear no relation to the irreversible change which takes place on treatment with acid or alkali, since the discrepancy between the forward- and back-titration curves was still present in these salt solutions. The lowering of the viscosity by the addition of salt must therefore involve a different type of physico-chemical change to that occurring on treatment with acid or alkali.

Experimental.

Apparatus.—The electrometric titrations were carried out according to the method described by Fletcher, Gulland, and Jordan (*loc. cit.*), and the titration curves were corrected for the titration of water at the extremes of pH by the method of Jordan and Taylor (*loc. cit.*). The solutions for titration contained 100 to 200 mg. of the samples in 20 ml. of water.

Preparation of Alkali-treated Samples of the Sodium Salt of Thymus Deoxypentose Nucleic Acid.—To a solution of sodium salt of deoxypentose nucleic acid (2 g.) in water (100 ml.), 0·5N-sodium hydroxide (25 ml.) was added with mechanical stirring. The solution then had a reaction of pH 12·30. 0·5N-Hydrochloric acid (24 ml.) diluted with water (51 ml.) was added slowly with rapid stirring to avoid precipitation. The pH was finally adjusted to 7·0. The solution was added immediately or at the time required to ethyl alcohol (750 ml.). The white granular precipitate (1·6 g.) was collected, washed with alcohol, and dried in a vacuum over phosphoric oxide.

Preparation of Acid-treated Samples of the Sodium Salt of Thymus Deoxypentose Nucleic Acid.—To a solution of the sodium salt of deoxypentose nucleic acid (2 g.) in water (150 ml.), 0·08N-hydrochloric acid (50 ml.) was added slowly with constant stirring, the final reaction being pH 3·1. 0·195N-Sodium hydroxide (24 ml.) was added slowly with stirring, and the reaction adjusted to pH 7·1 with dilute hydrochloric acid. The solution was added immediately or later as required to ethyl alcohol (750 ml.).

The white granular precipitate (1·6 g.) was collected, washed with alcohol, and dried in a vacuum over phosphoric oxide.

Preparation of Barium Thymate.—A solution of the sodium salt of thymus deoxypentose nucleic acid (15 g.) in water (400 ml.) was warmed to 80° and mixed with 10% sulphuric acid (30 ml.) at the same temperature. A white solid formed and redissolved in 5 minutes. The solution was maintained at 80° for 30 minutes. Silver sulphate (5 g.) was added and the suspension shaken for 1 hour, cooled in water and then to 0°, and mixed with a solution of barium acetate (20 g.) and barium chloride (5 g.) in water (30 ml.). Next day the supernatant was decanted, and the solid collected by centrifuge and washed repeatedly with 90% alcohol and then with 100% alcohol and ether. The product (4·6 g.) was dried in a vacuum over phosphoric oxide.

Analyses.—All samples were dried at 110° in a vacuum over phosphoric oxide. Details of the analytical methods are given in Part I (*loc. cit.*). Sodium salt of deoxypentose nucleic acid of calf thymus prepared by Professor Caspersson and deproteinised by us (see text), found: C, 35·5; H, 4·14; N, 15·4; P, 9·4; Na, 7·2 (colorimetric)%. Alkali-treated sodium salt of deoxypentose nucleic acid of calf thymus (respective values for three preparations), found: C, 35·3, 35·7, 36·1; H, 3·62, 3·83, 3·79; N, 15·4, 15·6, 15·5; P, 9·30, 9·63, 9·58; Na, —, 6·42, 6·28 (gravimetric) %. Acid-treated sodium salt of deoxypentose nucleic acid of calf thymus, found: C, 35·9; H, 3·76; N, 15·8; P, 9·41; Na, 6·33 (gravimetric) %. Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine, adenine, cytosine, and thymine deoxypentose nucleotides, *i.e.* $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$, the additional HONa of the terminal groups being ignored: C, 35·4; H, 3·4; N, 15·9; P, 9·4; Na, 6·95%. Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine and thymine, and 1·2 mols. of cytosine and 0·8 mol. of adenine deoxypentose nucleotides, *i.e.* $(C_{38\cdot8}H_{45}O_{24\cdot2}N_{14\cdot6}P_4Na_4)_x$, the additional HONa of the terminal groups being ignored: C, 35·4; H, 3·4; N, 15·5; P, 9·4; Na, 6·98%.

Barium thymate (respective values for two preparations), found: C, 27·1, —; H, 3·46, —; N, 5·44, 5·53; P, 9·47, 9·44; Ba, 23·3, 23·1%. Calc. for a molecule containing four atoms of phosphorus, 1·2 mols. of cytosine, 1·0 mol. of thymine, 2·22 atoms of barium and a terminal OH group to every five atoms of phosphorus, *i.e.*, $C_{29\cdot4}H_{40\cdot7}O_{25\cdot8}N_{5\cdot6}P_4Ba_{2\cdot22}$: C, 26·9; H, 3·10; N, 5·96; P, 9·44; Ba, 23·2%. Calc. for a molecule containing four atoms of phosphorus, 1·0 mol. each of cytosine, and thymine, 2·22 atoms of barium, and a terminal OH group to every five atoms of phosphorus, *i.e.*, $C_{29}H_{40\cdot1}O_{25\cdot8}N_5P_4Ba_{2\cdot22}$: C, 26·8; H, 3·08; N, 5·37; P, 9·56; Ba, 23·4%.

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