301. *Deoxypentose Nucleic Acids. Part VI. Electrometric Titration of the Acidic and Basic Groups of the Deoxypentose Nucleic Acids of Lamb Thymus and Herring Sperm.*

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The acidic and basic groups of the tetrasodium salts of the deoxypentose nucleic acids of lamb thymus and herring sperm have been titrated electrometrically, employing hydrogen electrodes. The conclusions drawn regarding the structure of these nucleic acids are very similar to those arrived at for the deoxypentose nucleic acid of calf thymus (*J.*, 1947, 1131).

In Part II of this series (Gulland, Jordan, and Taylor, *J.*, 1947, 1131) it was shown that the electrometric titration of a sample of the deoxypentose nucleic acid of calf thymus which had been prepared by a method which avoided the use of acid or alkali (Gulland, Jordan, and Threlfall, Part I, *J.*, 1947, 1129) showed anomalous behaviour. On addition of acid or alkali to a solution of the nucleic acid in water no groups were titrated at first between pH 5·0 and 11·0, but outside these limits there occurred a rapid liberation of groups which were titrated in the ranges pH 2·0—6·0 and pH 9·0—12·0. These groups were shown to be the $-NH_2$ and the $-NH-CO-$ groups of the purines and pyrimidines. Back-titration with either acid from pH 12·0 or alkali from pH 2·5 gave a titration curve which was different from that representing the initial titration but identical with the calculated curve. This behaviour was interpreted as showing evidence for the existence of hydrogen bonds between the $-NH_2$ and the $-NH-CO-$ groups of the nucleic acid macromolecule. In view of this interesting conclusion it was considered worth investigating deoxypentose nucleic acids from other sources. The acids chosen for the present investigation were those of lamb thymus and herring sperm.

Results.—The electrometric titration curves, which were corrected at the extremes of pH for the titration of the water by the method of Jordan and Taylor (*J.*, 1946, 994), are shown in Figs. 1 and 2. In both it is seen that the anomalous behaviour observed with the deoxypentose

nucleic acid of calf thymus (Gulland, Jordan, and Taylor, *loc. cit.*), and described above, occurs. The back-titration curves exhibit well-defined points of inflection in the region of pH 7·0, and show incipient points of inflection in the regions pH 11·8 and 2·5, corresponding respectively to 2·0 equivalents of alkali and 3·0 equivalents of acid for each four atoms of phosphorus.

FIG. 1.

The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of lamb thymus.

FIG. 2.

The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of herring sperm.

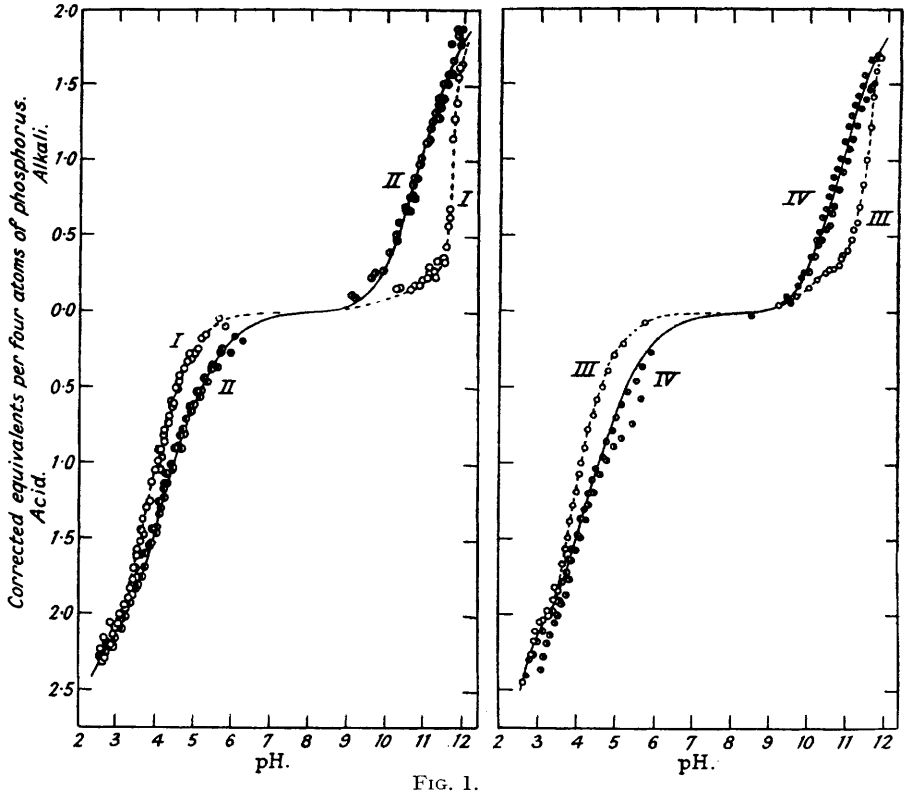

FIG. 1.

I. Titration with acid or alkali from pH 7·0, ○.

II. Back titration with acid from pH 12·0, ◑ *and with alkali from pH* 2·5, ◐. *The smooth curve through these points is calculated for* 1·2 *equivalents of* pK_a' 2·3, 0·8 *equivalent of* pK_a' 4·1, 1·0 *equivalent of* pK_a' 4·75, 0·2 *equivalent of* pK_a' 6·4, *and* 1·0 *equivalent each of* pK_a' 10·4 *and* pK_a' 11·4.

FIG. 2.

III. Titration with acid or alkali from pH 7·0, ○.

IV. Back titration with acid from pH 12·0, ◑ *and with alkali from pH* 2·5, ◐. *The smooth curve through these points is calculated for* 1·2 *equivalents of* pK_a' 2·3, 0·8 *equivalent of* pK_a' 4·0, 1·0 *equivalent of* pK_a' 4·8, 0·25 *equivalent of* pK_a' 6·4, *and* 1·0 *equivalent each of* pK_a' 10·4 *and* pK_a' 11·4.

Discussion.—The nature of the acidic and basic groups undergoing titration in the various pH ranges has been fully discussed in Part II (*loc. cit.*) and, although the same interpretation is made of the results recorded here, *viz.*, that the groups being titrated in the range pH 2·0—5·0 are the $-NH_3^+$ groups and those in the range pH 8·0 to 12·0 are the $-NH-CO^-$ groups, it is clear that slight differences in the pK_a' values of the amino-groups exist in the nucleic acids from different sources. Furthermore, the presence of only a small amount of a secondary phosphoric acid dissociation, when considered in conjunction with the sodium analysis of the sodium salt of the deoxypentose nucleic acid of lamb thymus, which shows that there are four atoms of sodium for every four atoms of phosphorus, is consistent with the view that this deoxypentose nucleic acid, like that from calf thymus, has a long unbranched chain structure.

The anomalous initial titration curves obtained (see Figs. 1 and 2) are important confirmatory

evidence for the results obtained with the deoxypentose nucleic acid of calf thymus, and show that the hydrogen-bonded structure exists in other deoxypentose nucleic acids which have been prepared in the manner described by Gulland, Jordan, and Threlfall (*loc. cit.*). An attempt is being made to prepare samples of nucleic acid which have been separated from the protein by a method other than the chloroform-gel method of Sevag, Lackmann, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425) in order to ascertain whether the hydrogen bonded structure is in any way dependent on the method employed in splitting the nucleic acid from the protein.

Experimental.

The electrometric titrations were carried out according to the method described by Fletcher, Gulland, and Jordan (*J.*, 1944, 33), 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 sodium salt of the deoxypentose nucleic acid of lamb thymus was prepared by Mr. C. J. Threlfall, B.Sc., by the method of Gulland, Jordan, and Threlfall (*loc. cit.*). The sodium salt of the deoxypentose nucleic acid of herring sperm was prepared by Professor M. Stacey by the method of Mirsky and Pollister (*Proc. Nat. Acad. Sci.*, 1942, **28**, 344) of which the method of Gulland, Jordan, and Threlfall (*loc. cit.*) is a modification.

Analyses.—All samples were dried at 110° in a vacuum over phosphoric oxide. The following results were obtained. Sodium salt of the deoxypentose nucleic acid of lamb thymus (Found : C, 36·3, 36·2; H, 4·2, 4·1; N, 15·6; P, 8·85; Na, 6·9, 7·0%). Sodium salt of the deoxypentose nucleic acid of herring sperm [Found : C, 34·3; H, 3·5; N, 15·3; P, 8·8. Calc. for a long polynucleotide consisting of the tetrasodium salt of tetranucleotides containing, on the average, 1 mol. each of guanine, thymine, cytosine, and adenine deoxypentose nucleotides, *i.e.*, $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$: C, 35·4; H, 3·4; N, 15·9; P, 9·4%]. The additional HONa atoms of the two terminal nucleotides of a straight-chain polynucleotide have been ignored since their contribution is negligible if the polynucleotide is large.

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