29. *Deoxypentose Nucleic Acids. Part VII.* A Re-examination of the Titration Curves of Sodium Deoxyribonucleate.*

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The anomalous electrometric titration of sodium deoxyribonucleate has been re-examined and the pG' values of the titrating groups have been calculated for the forward as well as the backward titration. An explanation for the change in the pG' values from forward to backward titration is given on the basis of the hydrogen-bond hypothesis. The difference between the backward titration curves from pH 2 and pH 12 is confirmed.

The application of electrometric titration to the study of nucleic acids has yielded significant results. Gulland, Jordan, and Taylor [1] found that the forward titration curves of sodium deoxyribonucleate of calf thymus from the neutral region to pH 2·0 and pH 12·0 differed from the curve obtained on backward titration from these extremes of pH. They suggested that this " hysteresis " effect might be due to the existence of hydrogen bonding between different titrating groups, which would influence the forward titration curves. This bonding is an integral feature of the structure recently put forward for nucleic acid by Crick and Watson.[2] The backward titration curves were interpreted by Gulland *et al.* as indicating one equivalent each of the amino-dissociations of guanine, adenine, and cytosine, and 0·25 equivalent of secondary phosphoryl dissociations for every 4 atoms of phosphorus. Similar conclusions have been reached by Signer and Schwander,[3] and by Cosgrove and Jordan,[4] for sodium deoxyribonucleate from other sources isolated by other methods.

Lee and Peacocke [5] avoided the drying procedure used by Gulland *et al.* and, employing more accurate analytical data, listed the pG' values of the guanine, adenine, cytosine, and secondary phosphoryl dissociations of sodium deoxyribonucleate from calf thymus, herring sperm, and wheat germ. They reported that there was a difference between the backward titration curves from pH 2·0 and pH 12·0, which Gulland *et al.* had found to be identical. In view of this, and of the added importance acquired by the hydrogen-bond hypothesis since its incorporation into the Crick and Watson structure, it was thought desirable to investigate the titration curves in greater detail, particularly the forward curve.

Results.—Fig. 1 shows a typical titration curve for sodium deoxyribonucleate. Fig. 2 shows in greater detail a number of forward titration curves in the acid region at different concentrations of sodium deoxyribonucleate.

Analysis of the titration curves in the acid region. It is possible [6] to represent the titration curve of a polybasic acid by a series of titration constants, G_1'. To achieve reasonably precise pG' values it is necessary to know accurately the analytical proportions of the different titrated groups. Such analytical data have only recently become available, through the chromatographic studies of Markham and Smith,[7] Wyatt,[8] and Chargaff, Zamenhof, and Green,[9] who conclude that the composition of deoxyribonucleic acid does not vary in different tissues of the same species. We have employed Wyatt's analytical figures for calf-thymus nucleic acid, 1·13 moles of adenine, 0·86 of guanine, 0·85 of cytosine, and 1·11 of thymine per 4 g.-atoms of phosphorus.

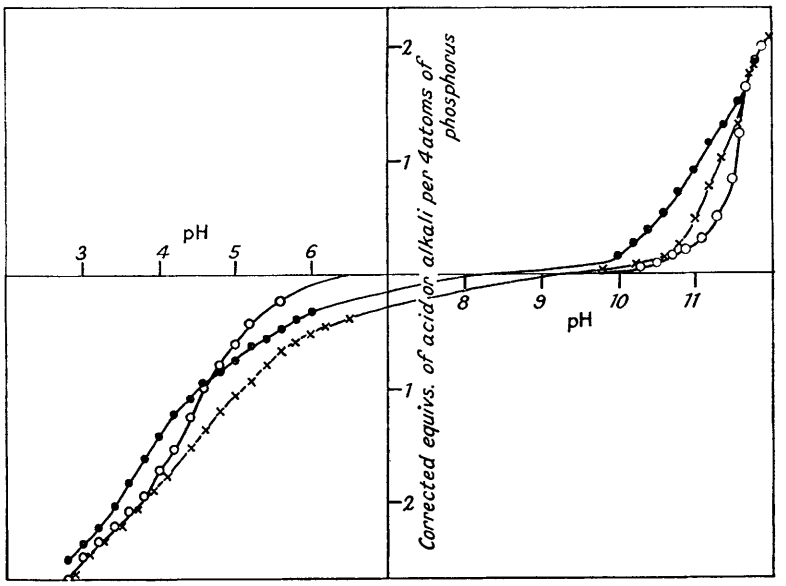
The detailed shape of the forward titration curves shown in Fig. 2 indicates that the pG'

* Part VI, *J.*, 1949, 1413.

[1] Gulland, Jordan and Taylor, *J.*, 1947, 1131.
[2] Crick and Watson, *Proc. Roy. Soc.*, 1954, *A*, **223**, 80.
[3] Signer and Schwander, *Helv. Chim. Acta*, 1949, **32**, 853.
[4] Cosgrove and Jordan, *J.*, 1949, 1413.
[5] Lee and Peacocke, *J.*, 1951, 2361.
[6] Simms, *J. Amer. Chem. Soc.*, 1926, **48**, 1239; Cohn and Edsall, " Proteins, Amino Acids, and Peptides, " Reinhold Publ. Corp., New York, 1943, p. 451.
[7] Markham and Smith, *Biochem. J.*, 1949, **45**, 294.
[8] Wyatt, *ibid.*, 1951, **48**, 584.
[9] Chargaff, Zamenhof, and Green, *Nature*, 1950 **165**, 756.

values of the bases titrating in the acid region are markedly different from those calculated from the backward curves. In particular, an inflection is present in the forward curves near pH 4, which suggests that the pG' values of guanine and adenine are appreciably further apart than

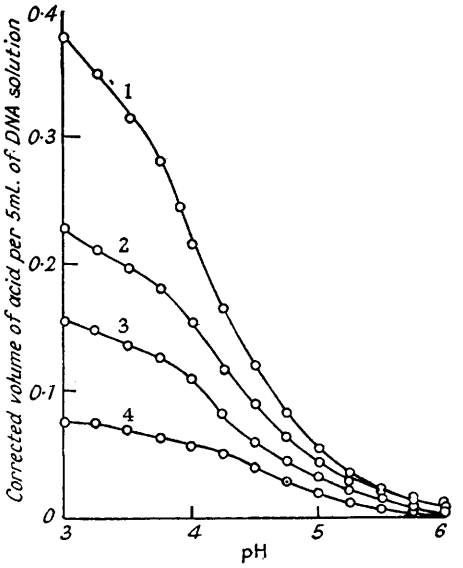
FIG. 1. *Titration of sodium deoxyribonucleate.*



○ *Titration from* pH 7·00 (*forward titration*).
× *Titration from* pH 2·50 (*backward titration*).
● *Titration from* pH 12·00 (*backward titration*).

FIG. 2. *Forward titration in the acid region at different nucleate concentrations* (C).

1, $C = 18{\cdot}75$;
2, $C = 11{\cdot}25$;
3, $C = 7{\cdot}50$;
4, $C = 3{\cdot}75$ (C *in* 10^{-4} *equiv. of* 4P *atoms per l.*).



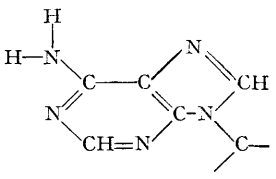
those calculated from the backward curves. This inflection occurs at higher pH values at lower concentrations of sodium deoxyribonucleate, which is to be expected from the effect of concentration on the pG' values reported in Part VIII (following paper). The initial steepness of the forward titration curves suggests that the pG' values of adenine and cytosine are much closer together than those calculated from the backward curves, which is fully supported by the

pG' values of the bases calculated from the forward titration curves, shown in the Table together with the corresponding pG' values calculated from the backward titration curves.

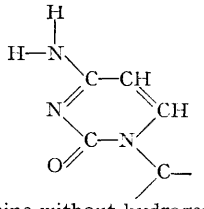
	Guanine	Adenine	Cytosine
pG' : forward titration	2·50	4·40	4·40
„ backward „	2·40	3·65	4·85

Discussion.—Interpretation of the forward titration curves. The difference between the forward and the backward titration curves has been ascribed to the existence of hydrogen bonds between the amino- and the carbonyl groups of the bases, which are irreversibly broken by acid or alkali. Guanine shows the same pG' value on both forward and backward titration, which suggests that the amino-group of guanine takes no part in hydrogen bonding. The structure proposed by Crick and Watson [2] for nucleic acid leaves it uncertain whether the amino-group of guanine takes part in a third hydrogen bond between guanine and cytosine. The titration results suggest that it does not.

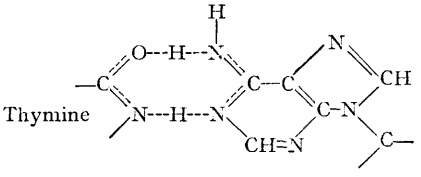
Adenine and cytosine exhibit the same pG' value on forward titration, but widely different values on backward titration, which may be explained on the hydrogen-bond hypothesis along the following lines. The annexed partial formulæ show adenine and cytosine in the normal state (*a* and *b*), and hydrogen bonded to thymine and guanine (*c* and *d*) as envisaged by Crick and Watson. The amino-nitrogen atoms of adenine and cytosine are in similar molecular environments in the hydrogen-bonded forms, as the eight-membered rings produced by the hydrogen bonding, which have to



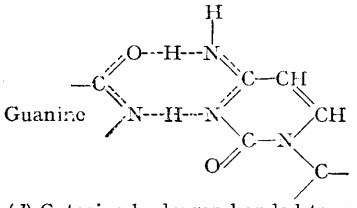
(*a*) Adenine without hydrogen bonds.



(*b*) Cytosine without hydrogen bonds.



(*c*) Adenine hydrogen bonded to thymine.



(*d*) Cytosine hydrogen bonded to guanine

be broken on titration, are the same in both cases. It is therefore to be expected that the pG' values of the amino-group in adenine and cytosine will be closer together in the hydrogen-bonded than in the non-bonded forms, since the hydrogen bonding will tend to mask the effect of the different resonating ring systems on the dissociation properties of the amino-group which is responsible for the different pG' values of adenine and cytosine. The fact that the pG' values are identical as calculated from the forward titration curve (Table 1) shows that the effect of the hydrogen-bonded rings is to mask completely this normal difference in the pG' values. It should be pointed out that these pG' values on forward titration refer to an irreversible change in the system.

Some confirmation of these views is to be obtained from the alkali-titration curves. These will not be discussed in such detail since they cannot be interpreted with confidence owing to the difficulty of making accurate calculations of the water correction at the highest pH. Nevertheless it is clear that the pG' values of the dissociating groups are much closer together on forward than on backward titration, and it is possible in consequence that this may be interpreted similarly.

The backward titration curves from pH 2 *and* pH 12. Fig. 1 shows that the backward

titration curves obtained by titration from pH 2 with alkali and from pH 12 with acid are not coincident, in agreement with Lee and Peacocke's results.[5] No satisfactory explanation of this effect has yet been found, but it is hoped that studies of the thermal and alkaline degradation of sodium deoxyribonucleate, and of the influence of pH on the streaming birefringence and electrophoretic mobility, now proceeding, may be of assistance in this.

Experimental

Preparation and Properties of the Sodium Deoxyribonucleate.—The samples of sodium deoxyribonucleate employed [G1(i) and G1(ii)] were extracted from calf-thymus tissue by the late R. H. Garner, B.Sc., using the procedure of Gulland, Jordan, and Threlfall.[10] A single specimen of nucleoprotein was obtained from the glands, and divided into 2 parts for the preparation of the 2 samples of sodium deoxyribonucleate. G1(ii) has a sedimentation constant (S°_{20}) in 0·2M-sodium chloride, extrapolated to infinite dilution of the nucleate, of $15{\cdot}4 \times 10^{-13}$, and a diffusion constant (D°_{20}) of $1{\cdot}06 \times 10^{-8}$ cm.^{2} sec.$^{-1}$. Combination of these results gives[11] a molecular weight of $7{\cdot}9 \times 10^6$. The rotary diffusion constant, and intrinsic viscosity at zero shear have also been measured for G1(i) and combined to give estimates of its molecular weight.[12] The fractional precipitation by hydrochloric acid and the streaming birefringence of G1(i) have also been studied.[13] The two samples gave identical titration curves within the limits of experimental error. They contained N, 15·7%, and P, 8·8%.

Preparation of the Solutions for Titration.—Stock solution (0·3%) of sodium deoxyribonucleate was prepared by dissolving the undried material, without stirring, in water of conductivity approx. 10^{-6} mho/ml., made in a still of the type described by Stuart and Wormwell,[14] and was kept at 0°. It has been shown [11,15] that many of the physical properties of sodium deoxyribonucleate alter when it is kept in solution, but the titration curves were reproducible within experimental error up to 6 weeks after dissolution. Measured portions of this stock solution were taken and made up to 5 ml. for titration.

Titration Procedure.—The cell consisted of a hydrogen and a standard silver–silver chloride electrode, the two compartments being connected with a saturated potassium chloride–agar salt bridge. The solutions were kept sufficiently stirred by the hydrogen bubbling over the electrode. Two platinum electrodes were kept in the solution and checked against one another from time to time during the titration. The E.M.F.'s were measured on a Tinsley potentiometer, and readings were estimated to be accurate to at least $\pm 0{\cdot}01$ unit of pH. 0·05N-Hydrochloric acid and -sodium hydroxide were used except when the volume to be added was such as to make the volume change too great to be corrected accurately by Taylor's method;[16] 0·5N-acid and -alkali were then used. The acid and alkali were added from an Agla micrometer syringe, and E.M.F. readings were taken after each addition of 0·01 ml. The reproducibility of titration curves obtained on solutions prepared from the same stock solution was $\pm 0{\cdot}01$ pH unit, except in the neutral region.

The concentration of nucleate in the stock solution was determined by analysis for phosphorus.[17]

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[15] Sadron, *Progr. Biophysics Biophys. Chem.*, 1953, **3**, 237;
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