[CONTRIBUTION FROM DEPT. OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, BERKELEY]

Deformation of Deoxyribonucleate. I. Titration and Optical Absorption Studies of the Effect of Temperature, Ionic Strength and *p*H on DNA Structure[1]

BY EDWARD L. DUGGAN, VINCENT L. STEVENS AND BENJAMIN W. GRUNBAUM

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DNA specimens have been prepared from thymus glands or chicken erythrocytes by a detergent method (Simmons) with the maintenance of at least 0.1 ionic strength at all steps. These preparations show different titration curves for the region of *p*H 8 to 2, according to the method of solution preparation from the lyophilized DNA. Thus, a 13-fold difference in titer (*p*H 4 to 8) is shown between DNA dissolved in 1 *M* KCl and DNA dissolved in water. Addition of dry KCl to the same molarity does not reduce the titer to that characteristic of the "salt-protected" DNA. The protected DNA shows minimal titer (*p*H 4–8) and minimal absorbance. The change from "salt-protected" DNA to deformed DNA is essentially irreversible. No terminal phosphate groups appear during deformation; the number of terminal phosphate groups is estimated to be less than 3 per 100 P. The critical range for significant change in titer on absorbance is between *p*H 3.5 and 3.0. Appreciable deformation of DNA occurs at *p*H 2.5, in agreement with the findings of many workers. Since the absorbance of the "salt-protected" DNA is lower than that of aqueous DNA samples, the absorbance increment from *p*H 6 to *p*H 2 is about 45%, in place of the 32% increment found by Thomas. The titrimetric data have been discussed in the light of the Watson–Crick structure of DNA, with emphasis upon the "free" amino groups.

Introduction

The manifest importance of deoxypentose nucleate (DNA) in genetic transmission or virus activity has overshadowed the existing gaps in the knowledge of the chemical behavior of this substance. This report is concerned with a re-examination of the acidic character of DNA by correlation of absorbance and titration data obtained with ionically protected DNA specimens.

It is impossible to compare the present titration data *in detail* with those obtained by the English groups[2–4] prior to the work of Cox and Peacocke,[5] since the latter workers are the first to use the point-by-point titration approach with the glass electrode. Cox and Peacocke[5] now realize that the hydrogen electrode is undependable for *p*H measurement under conditions of low buffer capacity (DNA, *p*H 4–9). These workers also emphasize that traces of alkali from glass containers may invalidate estimations of terminal phosphate titer in this *p*H region. This observation eliminates the possibility of calculation[6] of solvent titer (water blank), for the correction of titration curves of dilute solutes.

Our work has rested on the use of the glass electrode in the console titrator[7] which provides continuous recording of *p*H during titrations of solute and corresponding solvent. The method of curve-subtraction[8] is used to provide point-by-point values on corrected titration curves. These developments allow titration of milligram samples of DNA with minimum time and effort. DNA may only be investigated at microconcentration levels, in view of its high viscosity and present rarity.

The present article could be written in several forms of organization. Coherence of thought is favored by considering the possible forms of DNA as a groundwork for correlation of the data obtained. In the presently accepted structure of "undeformed" DNA,[9] the purine and pyrimidine bases are normal to the phosphoryl chain, while the sugar ring is in the plane of the chain. The following schematic diagrams are useful representations of the Watson–Crick structure of DNA (Fig. 1) and the "acid-deformed" DNA (Fig. 2). These diagrams suggest structures of the DNA without exact representation. In both figures, the sugar structures are represented by the intersecting lines. The base residues have been rotated through about 90° to allow the identification of each base as a square with an appropriate inscribed initial. The amino groups are presented as a circle tangent to the particular square. The circle represents $-NH_2$; the circle with an inscribed + sign indicates an $-NH_3^+$ group.

Watson-Crick Structure of DNA (pH 5-9)

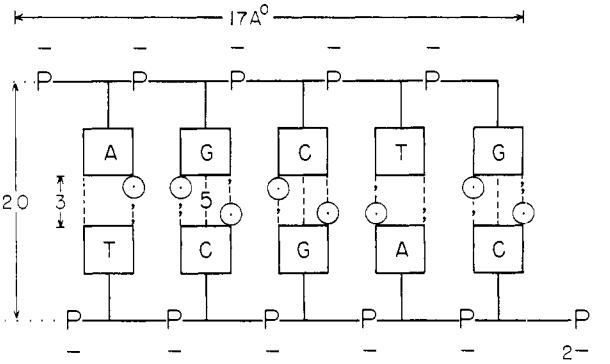


Fig. 1.—Schematic structure of undeformed DNA.

In Fig. 1, the various numbers are distances: a diameter of 20 Å., an axial length for five residues of 17 Å., the internuclear distance of 3 Å., and an estimated distance of 5 Å. between the centers of the amino groups on guanyl and cytidyl residues. The symbol P stands for a phosphoryl group, with one negative charge; it indicates a terminal phosphate group when two negative charges are shown. The dashed lines indicate hydrogen bonds, with no indication of the groups involved other than the

(1) Abstracted from the thesis to be presented by V. L. Stevens for the Ph.D. degree, 1957. Presented in part: *Federation Proc.*, **16**, 245 (1956). The work was supported by U. S. P. H. Grant C-2287 (C4).

(2) J. M. Gulland, D. O. Jordan and H. F. W. Taylor, *J. Chem. Soc.*, 1131 (1947).

(3) W. A. Lee and A. R. Peacocke, *ibid.*, 3361 (1951).

(4) D. O. Jordan, A. R. Mathieson and S. Matty, *ibid.*, 154, 158 (1956).

(5) R. A. Cox and A. R. Peacocke, *ibid.*, 2499 (1956).

(6) D. O. Jordan and H. F. W. Taylor, *ibid.*, 944 (1946).

(7) E. L. Duggan and V. L. Stevens, *Anal. Chem.*, **29**, 1076 (1957).

(8) V. L. Stevens and E. L. Duggan, *ibid.*, **29**, 1073 (1957).

(9) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

amino groups. The dotted lines indicate that the structure is merely a section of a long, continuous structure.

Figure 2 presents the structure which probably exists at low *p*H, in the first interval of acid treatment at low temperature. Proton uptake by DNA could begin at amino groups in imperfectly bonded regions. Electrostatic interaction between the resultant $-NH_3^+$ groups, and swelling due to solvation, could impose strain upon the remaining hydrogen bonds such that H-bond breakage and proton uptake could continue. The resulting structure would be an expanded helix, internally solvated, with the various amino groups in proton equilibrium with solvent, as governed by the individual *pK'* values.

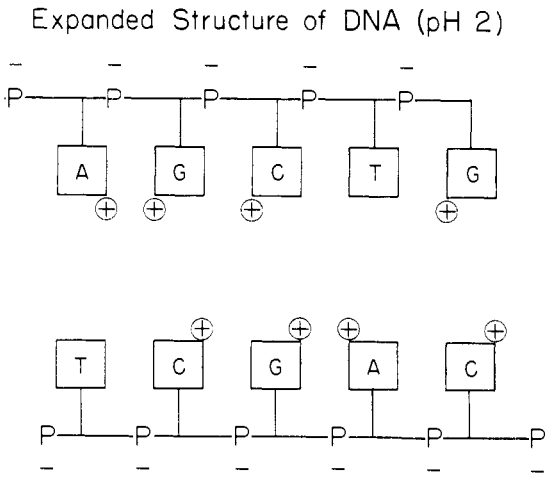


Fig. 2.—Schematic representation of immediate effect of acid on DNA structure.

It is possible that this expanded helical structure would be stable in solutions of high ionic strength, where the internal electrostatic effects could be minimized by the accumulation of "shielding" anion and additional electrolyte. Under conditions of low ionic strength, this structure should be only an intermediate in the disorganization (denaturation) of the DNA. One final structure might show interpenetration of base residues in such a way as to allow the close approach of the $-NH_3^+$ groups on one chain and the phosphoryl groups of other chains. All phosphoryl groups are considered to retain their single negative charges.

A second conformation[10] of DNA should exist at high *p*H. This structure would show internal solvation and expansion as described for the acid-deformed DNA (Fig. 2). The source of this expansion at high *p*H would be the conversion of the uncharged guanyl and thymyl residues into the corresponding anions by proton dissociation of their enol groups. The ionization and deformation should be complete above *p*H 12.[11] Since the molecule would acquire internal and external negative charges, the alkali-deformed DNA may exist for relatively long time intervals without further change in structure. Upon reneutralization, thymus DNA does not immediately return to its hydrogen-bonded organized structure, as shown by the "reversibility" of acid–base combination[2,5] of such deformed DNA samples. This reversibility may be a relative property, showing on the hour-long titrations of these workers, but not on the five minute titrations possible with automatic titrators. High ionic strength would be expected to stabilize the organized but expanded anion (*p*H 12), with unknown effect upon the reorganization of the alkali-treated DNA returned to neutral *p*H. Work is in progress to define these two effects.

In the following report we will describe recent data concerning the deformation of DNA by the simple process of dissolving dry DNA in water (very low ionic strength). In addition, we will summarize certain evidence from light absorption studies and *p*H titrations which support the conclusion that the structure (Fig. 2) does exist as an intermediate during the transition of the isolated DNA (Fig. 1) to deformed or degraded material.

Experimental

Titrimetric Studies.—The use of the console titrator[7] and curve-subtraction device[8] allows the rapid accumulation of corrected titration curves for 10 to 100 micromoles of an acid. Solutes are most easily investigated, if the titrations can proceed in samples of 1-ml. volume, with the acceptable 100 micromoles of solute. DNA studies do not permit the use of small volumes, since the corresponding acid groups would require less than 1 micromole of titrant for the full *p*H range (2–12). The results presented were obtained with 2.5 to 5 mg. of DNA in 10-ml. volume.

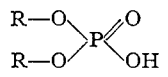
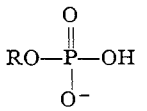
In an effort to standardize conditions for a series of titrations, weighed samples of lyophilized DNA (thymus or chicken erythrocyte) were dissolved by blending in a Teflon-glass homogenizer. It was observed that DNA dissolved rapidly in water, in dilute NaCl or sodium acetate, but slowly in 0.1 or 1 *M* KCl. Since these qualitative differences were found, titrations were performed on a series of DNA samples which had been prepared by direct solution into water or into electrolyte solution. A simple code was developed to classify the types of DNA solutions obtained. A titration is designated W-1 for the first titration of a DNA specimen dissolved in water. If repeated contact with acid and alkali occurred during a series of titrations of the same specimen, the titration curves could be designated W-1, W-2, etc. S-1 denotes a sample which was dissolved immediately in electrolyte of a stated molarity. If both designations are used (W-1, S-1), the sample has been subject to two titrations, the first in water, the second after the addition of dry salt. If WS-1 is used, the sample was dissolved in water, dry salt was added, and the single titration was accomplished.

The comparative titration curves of such varied solutions are shown in Fig. 3, for the *p*H interval of 4 to 8. It is apparent that the use of water as the solvent is responsible for the relatively great titer of the DNA sample in this *p*H region. Since it is difficult to compare the titer or shape of titration curves for an aqueous sample with a sample in 1 *M* KCl, the titration of the W-1, S-1 sample was completed. This titration evaluates the W-1 sample in a second titration after dry KCl was added to 1 *M* concentration. This sample had thus been exposed to low ionic strength conditions, titrated, adjusted to *p*H 3.5 (to avoid CO_2 uptake on opening the chamber), the KCl was added, and the second titration was accomplished. The three titer values for *p*H 4 to 8 increase from the S-1 sample (3 units) to the W-1, S-1 sample (14 units) to the aqueous sample (40 units). Thus the mode of preparation of the DNA samples has resulted in a 13-fold difference in titer of the groups releasing protons in this *p*H region. These groups had accepted protons by the initial adjustment of each solution to *p*H 3.5. A certain amount of "salt-return" toward the state of the sample initially dissolved in KCl is shown by the decrease of titer of the W-1, S-1 sample from the titer shown by the same DNA sample in the first titration (W-1). As a measure of the new groups appearing during the treatment, the titer difference between the two titrations in 1 *M* KCl may

(10) R. Lumry and H. Eyring, *J. Phys. Chem.*, **58**, 110 (1954).
(11) J. Shack and J. M. Thompsett, *J. Biol. Chem.*, **197**, 17 (1952).

TABLE I

THE ACIDIC GROUPS OF CALF THYMUS DNA

		G–NH_3^+	A–NH_3^+	C–NH_3^+	
pK', lit.[14]	...	2.3	3.7	4.2	6.0
pK', proposed	2.5	3.0	5.0	5.0[13]	6.0
Residues/100 P[15]	100	21	29	21	0
Sum of residues	171	71	50	21	0

be calculated. The difference curve obtained by subtraction of curve S-1 from curve W-1, S-1 indicates that no new groups appear in the region of pH 6 to 8, since the curve has zero slope in this region. The complete titer increment is found in the region of pH 4 to 6, which indicates that the new groups have pK' values of 5 or less. Similar results were obtained, using DNA from chicken erythrocyte.[12]

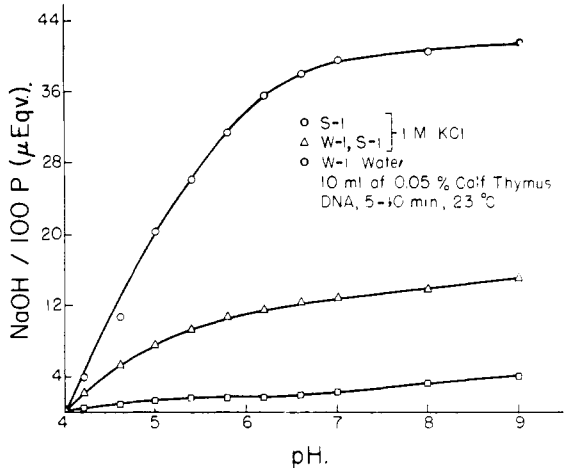


Fig. 3.—Effect of ionic history and ionic strength on titrations of calf thymus DNA. The points are obtained by interpolative subtraction of continuous solvent curves from sample curves.[8]

Various workers agree that terminal phosphate, if present, should show its secondary dissociation in the region of pH 4 to 8, with a pK' of about 6.5. Considering the lack of slope in the pH 6 to 8 region for either titration in 1 M KCl, as well as the difference curve, it seems inescapable that only titrations of the S-1 type can be used for estimation of terminal phosphate. The extremely low titer of such samples (3 microequivalents per 100 microgram-atoms of phosphorus) is in accord with the accepted high molecular weight of this substance. The titer value sets an *upper limit* for the number of terminal phosphoryl groups present. The titer in this pH interval could also include a fraction of the dissociation step of cytidyl-NH_3^+ if the pK' (5.0) found for this group in apurinic acid[13] is used. Calculations based on this pK' value, and the content of this residue (21 groups per 100 P) lead to the estimation that cytidyl-amino groups, if free, could contribute 19 units of titer in this pH region (4–8). The enolic dissociations of guanyl and thymyl residues could also require fractional units of titer, if the pK' value of these acids is taken as 10.0.

Similar titrations of DNA following varied solution procedures are given in Fig. 4. These DNA samples were dissolved in water or in 0.1 M sodium chloride; the titrations extended from pH 9 to 2.5. Titration of groups on the W-1 sample proceeds from pH 5.5 to 2.5, with double uptake of hydrogen ions compared to the two titrations at 0.1 ionic strength. The two samples titrated at this ionic strength show better agreement in location of titer increments and in ultimate titer. The S-1 type of titration would still be the standard of comparison. The WS-1 sample providing the titration curve differs in one important respect from the sample termed W-1, S-1 (Fig. 3). The WS-1 sample has *not* been exposed to alkali (pH 9) or acid (pH 4) prior to addition of dry salt. The W-1, S-1 sample was subject to one titration before addition of salt. On this basis, one would expect that the WS-1 sample is less deformed since salt was added before any exposure to these moderate pH extremes. Qualitatively similar results were obtained on titration of chicken erythrocyte DNA; this DNA became insoluble at pH 2.5 during titration.

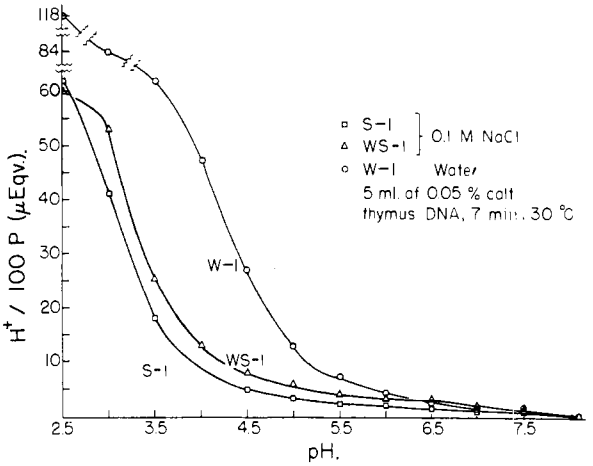


Fig. 4.—Effect of ionic history and ionic strength on the titration of DNA from pH 8 to 2.5.

Table I presents the quantity and possible pK' values for the various acidic groups of DNA which *could* react with alkali during titration from pH 1 to 8. The residues are shown as partial formulas or as G–NH_3^+, etc., where the letters designate guanyl, adenyl or cytidyl residues. The numbers of residues are listed as moles per 100 g.-atom of phosphorus (this assumes 1 residue for each P). The summation of residues begins at the right of the table, for the reference pH of 8 where these dissociations are presumed complete. This sum is shown as one possible means of comparison of observed titer values with the potential titer. The thymyl residues (29/100 P) are not listed since this pyrimidine has no dissociating group in this pH region.

All calculations of titer proceed by substitution of the desired pH value and an individual pK' value into the common buffer equation

$$pH - pK' = \log [S]/[A]$$

where S is the salt or the conjugate base, and A is the acidic form of the group. The analytical values for the groups are the sum of S + A. Knowing the S/A ratio and the sum, the quantity of S or of A may be calculated. Total titer for the DNA is the sum of the increments of A for all groups.

Table II presents a comparison of calculated and observed titer values for the DNA. Calculations by method 1 follow the described routine, using the literature pK' values; those by method 2 use the proposed pK' values. The esti-

(12) Prepared by Method D, private communication from N. S. Simmons. The method is briefly described in the second article of this series.

(13) E. Hurlen, S. G. Laland, R. A. Cox and A. R. Peacocke, *Acta Chem. Scand.*, **10**, 793 (1956).

(14) P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **70**, 327 (1926).

(15) E. Chargaff and R. Lipshitz, THIS JOURNAL, **75**, 3658 (1953).

mates of titer given in Table II are recognized as first approximations, since a rigorous treatment of the titration behavior of DNA is not possible at present. This ultimate treatment must assess the statistical interactions of groups, the electrostatic interactions between the ammonium acids at the close distances implied by the structure,[9] as well as the general effect of the large negative charge of the molecule upon the dissociations from all groups.[15a,15b] The calculated pK' values of the ammonium-type acids must be raised by the high negative charge on the macro-ion, for low ionic strength. The pK' values of these acids may trend toward the value for similar groups in mononucleotides, at high ionic strength.

TABLE II

COMPARISON OF CALCULATED AND OBSERVED TITERS OF CALF THYMUS DNA TITRATIONS

	Titers, calcd. or obsd. (μequiv./100 μg.-atom P)		
pH 4–8	Method 1	Method 2	Obsd.
S-1, 23°, 1 *M* KCl	14	51	3
S-1, 30°, 0.1 *M* NaCl	14	51	9
W-1, 23°	14	51	40
W-1, 30°	14	51	48
pH 2.5–8			
S-1, 30°, 0.1 *M* NaCl	58	115	63
W-1, 30°	58	115	118

All calculations imply that the total number of the acidic group is available for equilibration with the hydrated protons. Using the terminology of the titer calculation methods, S + A must be the analytical value, so that rapid establishment of S/A ratios (and *p*H) are possible. The data presented graphically in Fig. 3 indicate that S + A is changing with the conditions of solution of the DNA, so that S/A values merely show equilibration of the available $-NH_2$ groups, *i.e.*, those not presently held in hydrogen bonds (Fig. 1).

The difference in behavior of the salt-protected DNA (S-1) and that of the aqueous DNA (W-1) is shown as a titer difference in the figures. The data are subject to three interpretations: (a) The pK's of available groups have decreased sufficiently to carry the titration curve out of the selected *p*H range, as the ionic strength is increased. (b) Available groups are reacting with hydrogen ion, and additional groups are being exposed rapidly during the titrations. (c) The samples differ in number of free groups (S + A); these groups are able to equilibrate with hydrogen ion.

If the three curves (Fig. 3) are plotted on three ordinate scales to bring all into coincidence at *p*H 7, all curves have similar shape. The *p*H displacement from the W-1 to the two S-1 curves is 0.35 *p*H unit to the left. If such *p*H displacement (correction for ionic strength) is applied to the W-1 curve, the total titer value would be reduced only to 32 units. The ordinate values of the three, the corrected W-1 curve and the two S-1 curves, would remain in the qualitative relations shown in the figure. This evidence eliminates the first interpretation as a possibility. Similarly, the change in character of the titration of the W-1 sample after salt addition (now W-1, S-1) may not be explained on the basis of a pK' shift.

The second possibility, that the S-1 type of titration would change to that of the W-1 sample, on continued exposure to the acid (*p*H 3.5) and alkali (*p*H 9), is eliminated by the following observations. The titration curves may be repeated several times, even on the same sample, without significant increase in titer of the low-titer samples. Identical titrations performed at 60° on similar samples yielded titer values of 38, 26 and 7, for the W-1, W-1/S-1 and S-1 samples, respectively. Return toward the protected form of DNA has diminished, while "salt-protection" is still significant.

The third possibility, that the samples differ only in the number of free amino groups, is preferred, since the titration data are explained without the necessity of invoking a pK shift of 0.35 unit for the data of Fig. 3 and 1 *p*H unit for the data of Fig. 4.

(15) (a) T. Hill, *Arch. Biochem. Biophys.*, **57**, 229 (1955); (b) R. A. Cox and A. R. Peacocke, *J. Chem. Soc.*, 2499 (1956).

Gulland, *et al.*,[2] first observed the hysteresis character of the titration of DNA with acid (*p*H 8–2), followed by titration with alkali (*p*H 2–8). The first and second titrations differed with respect to location on the *p*H scale. These workers found a rough agreement between calculated titration curve and the second experimental curve (*p*H 2–8), on the basis of the known pK' values of the amino groups of the nucleosides or nucleotides. This second behavior was termed reversible, since the third and succeeding titrations retraced this curve. Thus reversible equilibria with hydrogen ion characterize the titration of acid-deformed (or alkali-deformed) DNA, but not the initial reaction of the isolated well-organized DNA.

Table II allows the observation that the observed titers are small with respect to all calculated values for the S-1 type of DNA sample. For the *p*H 4–8 interval, the observed titers (W-1) agree more closely with the calculated value by method 2. This agreement may be fortuitous, since the numerous phosphoryl groups should possess a pK' value relatively independent of the internal structure of the DNA, so that several units of titer must represent phosphoryl group dissociations in the *p*H 4 to 5 interval.

The observed titers differ twofold (63 units and 118 units) between the S-1 sample and the W-1 sample, for the titration from *p*H 8 to 2.5. Calculation by method 2 is still valid since agreement is obtained between calculated and observed titer for the W-1 sample. The S-1 sample would then show a deficit of 55 units of titer between calculated and observed values; this deficit is probably due to a small fraction of unavailable amino groups and a decreased pK' of the phosphoryl groups. Method 1 includes no estimate of phosphoryl titer, an acceptable approximation for *p*H 4–8 but not for the region of *p*H 2.5–4. Thus this method would provide a low potential titer value at *p*H 2.5.

Regardless of the method of calculation, it is apparent that all groups of the DNA are not titrated in any sample. It is probable that the DNA retains a net negative charge at all *p*H values above *p*H 3, at low cation concentration. Considering the variation of total titer for the two regions as a function of the method of solution of the DNA, it seems apparent that the phosphoryl groups do not titrate appreciably in the interval of *p*H 4–8. Also, it appears that titration to *p*H 2.5 in the presence of 0.1 *M* NaCl does not measure any appreciable fraction of the phosphoryl groups. This implies that the pK' value of the phosphoryl dissociation is less than 2.5, at 0.1 ionic strength. Evidence from the absorbance studies indicates that the ionic protection of DNA does not exist at *p*H 2.5; the maximal absorbance changes indicate complete availability of nuclear amino groups.

Absorbance Studies.—The investigation of the various DNA preparations by optical techniques remains important, since absorbance measurements provide universal correlation between laboratories for individual DNA samples.

It was of interest to examine the behavior of DNA samples with respect to change of absorbance with *p*H. Thus, the transformation of hypochromic DNA to hyperchromic DNA could be correlated with the range of proton uptake, from the recorded *p*H titrations. One must recognize that there is an inevitable 25-fold difference in the concentration level of the DNA during titration for volume and *p*H recording (0.5 mg. DNA per ml.) and the level during titration in the cuvet (0.02 mg. DNA per ml.).

The data for the absorbance–*p*H behavior of various DNA samples are shown in Fig. 5. The code summarizes the various procedures from left to right, each symbol having the same meaning as in the titration section. Thus, W indicates that the DNA was dissolved in water. Addition of dry KCl to 0.1 *M* concentration after the solution step yields a WS sample. Dissolving dry DNA in 0.1 *M* KCl provides an S sample. Heating in water is designated by WH; heating of the WS solution, WSH.

Inspection of the figure indicates significant results of the solution and heating procedures. It is apparent that stable absorbance for a broad *p*H range is obtained for the DNA dissolved in salt (S). This behavior indicates a *p*H-stable hypochromic form of the DNA. This sample showed a rapid rise in absorbance between *p*H 3 and 2, corresponding to a 45% increase, from a molar absorptivity, A(P),[16] of 6500 to a value of 9400.

(16) E. Chargaff and S. Zamenhof, *J. Biol. Chem.*, **173**, 327 (1948).

The upper limit for the absorbance behavior of the DNA is shown by the curve WH, where the A(P) is close to 9000 at all measured *p*H values. This DNA may have been deformed to a maximal extent so that it now exists in the hyperchromic state even at neutral *p*H values and room temperature. This finding was unexpected since the thermal destruction of hydrogen bonds might be expected to be reversible during standing at room temperature. This finding confirms the similar observation of Thomas[17] that heating in water brings DNA to high absorbance, with little change in this value during acid additions. The structure of the DNA corresponding to the heated samples may be considered to be represented by that shown in Fig. 2 with the amino groups uncharged. The maintenance of the hyperchromic form may have been favored by the low DNA concentration during heating and cooling. Whether ester bonds are broken during heating in water[18] and whether actual single chain DNA exists after such treatment remains for further investigations.

A reformation of hydrogen bonds following solution of DNA in water is indicated by the downward displacement of the WS curve with respect to the W curve, even though a difference remains between WS and S samples. The protection of the DNA during heating is shown by the relative location of the W and WH curves, or the WS and WSH curves. From other work,[17,19] it is apparent that the magnitude of deformation obtained on heating a DNA sample is a function of the ionic strength. Lawley's[19] detailed study of the effect of heating on DNA indicates that samples containing 0.1 m*M* sodium ion are deformed at lower temperatures (50°) and the absorbance rise is maintained upon cooling. Samples with 10 m*M* sodium ion require heating at 70 to 80°, and show a greater return toward the absorbance of unheated DNA after the sample cools to room temperature.

Discussion

Abundant evidence has been reported for the effect of ionic strength upon the absorbance of DNA specimens during heat, or acid treatments. The major findings contributed by this work indicate that the DNA specimens show parallel changes in titer and absorbance following brief storage in water, which are permanent since added salt will not allow a return to the status of a DNA sample initially dissolved in salt solutions.

Considering these findings, we have concluded that there is no present need to invoke *pK'* shifts in explanation of titer changes in a given *p*H range. Rather, one can consider that the natural or induced imperfections in hydrogen bonding of the DNA sample allow equilibrium with hydrogen ion, while the bonded sections do not allow a similar reaction. The structures given in Figs. 1 and 2 may be considered to represent limits of possible structures for isolated DNA, and deformed DNA, respectively. The structure first given would be expected to be hypochromic, hypotiter and relatively rigid. That given in Fig. 2 would be hyperchromic, hypertiter or titrated, and flexible. It is interesting that the results from titrations, and measurements of intrinsic viscosity[20] indicate that *p*H 3 is the stable limit for DNA. Below *p*H 3, a precipitous fall in viscosity occurs, as well as a decrease in molecular size (light-scattering) with no change in molecular weight.[20]

The alkali and alkaline earth cations protect DNA from deformation, as evidenced by the data reported here, as well as the work of others.[17,19,21] Evidence to be presented in a later article indicates that lead ion is capable of precipitation of heat-treated DNA. In the presence of the lead ion, the DNA is deformed at lower temperatures. The actual structure of the lead DNA salt is unknown.

Materials and Methods

DNA Source and Method of Preparation.—DNA was prepared from calf thymus by the method B of Simmons[22] which involves the use of sodium xylenesulfonate and of alcohol precipitation. Analysis: 8% P; N/P = 1.71. RNA analysis: 24 hr. hydrolysis in 1 *N* NaOH followed by chromatography in isobutyric acid:ammonia[23] showed no ultraviolet absorbing spots except at the orign. Protein: negative by the method of Sevag, *et al.*[24]

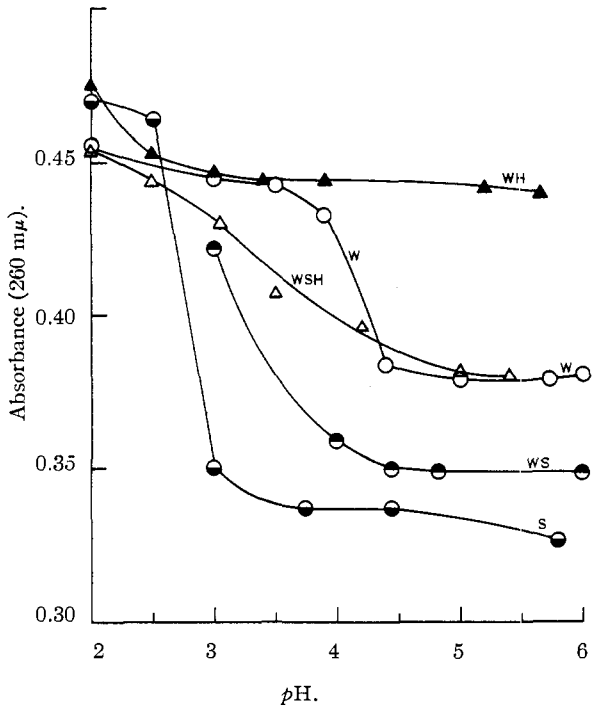


Fig. 5.—Effect of solution process or heating upon the absorbance–*p*H behavior of calf thymus DNA. The identification of curves is given in the text.

Automatic Potentiometric Titration.—Apparatus is described in another article.[7] Five and ten-ml. samples were used; the DNA concentration varied from 0.2 to 0.5 mg./ml. Titrant concentration was 0.5 *M* sodium hydroxide or hydrochloric acid.

Cuvet Titration Technique.—Titrations of DNA in which *p*H is plotted *versus* optical absorbance change were performed with the following technique. A quartz cuvet is cut off at about one half its height and employed in the Lowry attachment for the Beckman DU spectrophotometer. A tiny magnetic stirring bar is inserted in the cuvet, which is placed in the Lowry cuvet holder at such a height that the stirring bar does not interfere with the passage of light through the cell. After absorbance measurement the cuvet and holder are removed from the spectrophotometer and placed over a magnetic stirrer. A syringe buret with a fine polyethylene tip delivers titrant. *p*H is measured with a Beckman model G *p*H meter, equipped with a 290-1 glass electrode and a standard calomel electrode which is connected to the sample solution *via* a flexible salt bridge. Titrant is added with stirring, the *p*H is taken and the absorbance is measured. This type of point-by-point titration requires about 2 minutes per point. The sample is usually a 1-ml. aliquot of a DNA solution containing of the order of 0.02 mg. DNA/ml. The titrant used was 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide.

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