at the beginning of the exchange reaction, reached a maximum in intensity and then diminished as deuteration neared completion. The two bands are assigned as the asymmetric B–H′$_{single}$ and B–D′$_{single}$ vibrations of a partially deuterated bridge supporting previous assignments[12–14] for such partially deuterated bridges.

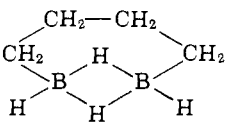
TABLE II

INFRARED SPECTRA[a] OF 1,2-TETRAMETHYLENEDIBORANE (I), PARTIALLY DEUTERATED 1,2-TETRAMETHYLENEDIBORANE (I_d) AND 1,2-(1′-METHYLTRIMETHYLENE)-DIBORANE (II)

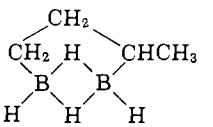
Compound I	Compound I_d	Compound II
		2950s
2930s	2930s	
2890sh	2890sh	2900
2840w	2840w	
2510s	2510mw	2530s
	1890s	
	1640m	
1580vs	1580w	1510vs
1460w	1460m	1470sh
1420m	1410m	1430sh
	1350m	
1300mw	1300m	
		1260m
	1230mw	
	1170vs	
1120s		1130ms
		1060s
	1090m	
	1030ms	
	930m	
870m		
	830m	

[a] Perkin–Elmer model 21 double-beam spectrophotometer equipped with sodium chloride optics.

In view of other additions of diborane across double bonds, two structures involving no carbon–carbon rupture came into consideration



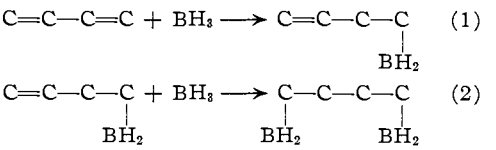
I



II

Strain considerations militate against organodiboranes with only two carbons in the ring. The total combined spectral evidence along with the previously mentioned hydrolysis and oxidation of I to 1,4-butanediol established I as the compound with the B_2C_4 ring. The B_2C_3 ring structure shown above is therefore assigned to represent compound II. Consideration of bond angles and interatomic distances decrees *cis*-type structures (*i.e.*, the BH groups are *cis* to each other). Therefore, the five-membered ring would be expected to be planar with some strain involved, while the six-membered ring would be non-planar and involve little or no strain.

These results show that definite compounds are formed, in addition to polymeric materials, in the reaction between diborane and butadiene in the gas phase. The addition of diborane is primarily to the 1,4-positions of 1,3-butadiene, but a small amount of addition to the 1,3-positions also takes place. The reaction most likely occurs as addition of BH_3, *viz.*



The intermediate product may then cyclize to give the more stable form of 1,2-tetramethylenediborane.

Acknowledgment.—The authors wish to thank Dr. R. E. Williams for assistance with the n.m.r. spectra.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE SPECTROSCOPY LABORATORY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

Hydration of Deoxyribonucleic Acid. I. A Gravimetric Study[1]

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The weight of water adsorbed by solid sodium and lithium deoxyribonucleate was determined as a function of relative humidity at 21°. An equation of the BET type fits the data between 0 and 80% relative humidity. From the values of the BET constants it is concluded that two molecules of water are strongly bound to each phosphate group, with an energy about 2 kcal. higher than that for the adsorption of further water molecules. The disappearance of adsorption hysteresis, negative deviation from the BET equation, and the sharp onset of swelling, all of which occur above 80% relative humidity, indicate that at about this point water fills completely the void spaces in the DNA structure.

Introduction

The structure of deoxyribonucleic acid has been found to be highly sensitive to the humidity of the surrounding atmosphere.[3] Wilkins and coworkers[4–6] have reported at least four different types of X-ray diffraction patterns from DNA fibers

(1) This work was supported by Grant No. A-2262(C3) from the National Institutes of Health, Public Health Service.

(2) Atlantic Regional Laboratory, National Research Council, Halifax, Canada.

(3) R. E. Franklin and R. G. Gosling, *Acta Cryst.*, **6**, 673 (1953).

(4) M. Feughelman, R. Langridge, W. E. Seeds, A. R. Stokes, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, R. K. Barclay and L. D. Hamilton, *Nature*, **175**, 834 (1955).

(5) R. Langridge, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *J. Mol. Biol.*, **2**, 19 (1960).

(6) D. A. Marvin, M. Spencer, M. H. F. Wilkins and L. D. Hamilton, *ibid.*, **3**, 547 (1961).

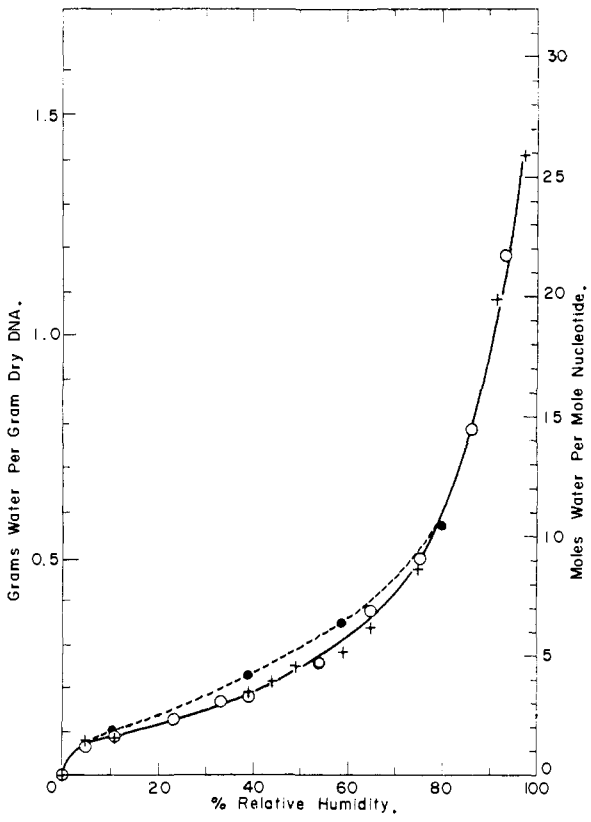

Fig. 1.—Adsorption (open circles) and desorption (full circles) of water by a sample of calf-thymus NaDNA; adsorption of water by a sample of salmon-sperm NaDNA (crosses).

and interpreted them as distinct molecular configurations, each stable over a limited range of relative humidity (r.h.). Further changes in structure have been reported to occur in dry fibers.[3] In view of the importance of this problem, we have undertaken a study of hydration of DNA using several experimental techniques. The present paper deals with a gravimetric study of DNA and of related compounds. The gravimetric data give directly the number of molecules of water bound to DNA as a function of r.h., and allow a rough estimate of the binding energy. In a following paper[7] we shall present the infrared evidence on the molecular sites of DNA to which water is bound at various values of r.h., and on the nature of the binding. A study of infrared and ultraviolet dichroism of oriented samples of DNA has also been carried out and will be presented together with some conclusions concerning the structural changes occurring in DNA as a result of hydration and dehydration.

Experimental

Samples of DNA were purchased as sodium salts (NaDNA) from commercial suppliers: calf-thymus DNA from the Nutritional Biochemicals Corporation, Cleveland, O., and salmon-sperm DNA from the California Corporation for Biochemical Research, Los Angeles, Calif. These samples, stated by the suppliers to be free of protein and found by us also to be free of chloride ion, were further screened for these studies by spectroscopic means. The samples used were those which could be stroked into highly oriented films, as shown by polarized infrared and ultraviolet spectra, and which exhibited the full extent of ultraviolet hypochromism characteristic of undenatured DNA. The lithium salt (LiDNA) was prepared by dissolving 0.1 g. of NaDNA in 25 ml. of 0.01 *M* LiCl solution, adding an equal amount of 2.0 *M* LiCl, equilibrating overnight, then precipitating with isopropyl alcohol. The precipitate was washed for several days with 80% ethyl alcohol until free of chloride ion.

Samples weighing about 0.1 g. and contained in Pyrex weighing bottles were exposed to atmospheres of constant r.h. in evacuated glass desiccators and weighed to 0.0001 g. daily until the weights remained constant for about a week. Blank weighing bottles were used to determine and allow for the amount of adsorption of water on glass, which was appreciable at 92% r.h. (about 0.0004 g. or 0.4% of weight of water). The temperature was kept at 21 ± 0.5°. The constant r.h. was maintained by a saturated solution of an appropriate salt contained in a flat dish. The r.h. values for most of the salt solutions were derived by a small extrapolation to 21° from the data of Wink and Sears[8] and are probably good to better than ±0.5% r.h. The accuracy of several r.h. values taken from the "International Critical Tables" (marked with an asterisk in Table I) is probably ±2% r.h. or better.

TABLE I

AVERAGE ADSORPTION OBSERVED FOR NaDNA (A_{obsd}) IN MOLES OF WATER PER MOLE OF NUCLEOTIDE AND VALUES CALCULATED FROM EQ. 1 (A_{calcd}) FOR $B = 2.2$ AND $C = 21.0$

Salt solution	% r.h.	A_{obsd}	A_{calcd}	$A_{obsd} - A_{calcd}$
Sodium hydroxide	5.0[a]	1.32 ± 0.09	1.21	+0.11
Lithium chloride	11.1	1.68 ± 0.19	1.79	− .11
Potassium acetate	23.0	2.32[b]	2.46	− .14
Magnesium chloride	33.1	3.06[b]	3.00	+ .06
Chromic oxide	38.8	3.37 ± 0.20	3.34	+ .03
Potassium nitrite	48.8	4.23 ± 0.44	4.09	+ .14
Magnesium nitrate	54.0	4.71[b]	4.60	+ .11
Sodium bromide	59.0	5.33 ± 0.22	5.19	+ .14
Sodium nitrite	65.2	6.09 ± .46	6.17	− .08
Sodium chlorate	75.0[a]	8.42 ± .27	8.66	− .24
Ammonium sulfate	80.3	10.15 ± .15	11.04	− .89
Potassium bromide	84.0[a]	10.53 ± .35	13.62	−3.09
Potassium chromate	86.6	13.12 ± 1.37	16.30	−3.18
Sodium bromate	92.0[a]	20.04 ± .55	27.39	−7.35
Ammonium dihydrogen phosphate	93.2	20.73 ± 1.04	32.20	−11.47

[a] r.h. values from "International Critical Tables." [b] Measurement on one sample only.

The dry weight was obtained by drying the samples to constant weight in vacuum at room temperature. This was shown to be sufficient by the fact that drying at 100° produced no further loss in weight. The samples were hydrated to constant weight at fixed r.h. Usually the equilibrium of hydration was approached from lower values of r.h., but some points were obtained by approaching the equilibrium from higher r.h. values. Weight *vs.* r.h. curves obtained by approaching the equilibrium from lower r.h. are referred to below as *adsorption* curves and those from higher r.h. as *desorption* curves.

Results

The adsorption and desorption curves for a typical sample of calf-thymus NaDNA are plotted in Fig. 1. In the range of 10 to 80% r.h. the desorption curves of all samples lie above the adsorption curves. This adsorption–desorption hysteresis appears genuine, not being eliminated by a prolonged equilibration of many weeks. At its maximum, the hysteresis amounts to a little over 1 water molecule per nucleotide. Within either the adsorption or the desorption branch the results for a single specimen were reproducible to about 0.1 water molecule per nucleotide at low r.h. and to about 0.5 water molecule at high r.h. For many

(7) In preparation.

(8) W. A. Wink and R. R. Sears, *Tappi*, **33**, 96A (1950).

different specimens the results show a spread of ± 0.2 to ± 1 water molecule per nucleotide. Within this spread there is no difference in the adsorption (or in the desorption) of water by calf-thymus and by salmon-sperm samples. In Table I the average adsorption values in moles water per mole nucleotide are recorded from six salmon-sperm and two calf-thymus specimens of NaDNA, together with the average deviation. The adsorption curve of LiDNA lies slightly higher than that of NaDNA below 30% r.h. This difference seems to be real, although only a little outside the spread of the data. The two curves appear to cross above 30% r.h., but this may be spurious. Adsorption of water on both NaDNA[3,4] and LiDNA[5] has been measured at several isolated r.h. values by previous investigators in the course of their X-ray diffraction studies. Their results are in general agreement with the present data.

Discussion

Comparison with Model Compounds.—Concurrent study of hydration of constituents of DNA and related compounds, which we intend to report separately, has shown that as a rule purines and pyrimidines, as well as their nucleosides and nucleotides, do not form stable hydrates in the 0 to 92% r.h. range at room temperature. On the other hand, the salts of the nucleotides, as well as other compounds containing the ionic phosphate group, form a series of distinct hydrates, as shown in the adsorption curve for monosodium cytidine-5′-phosphate (Fig. 2). It therefore appears that the oxygen atoms of the carbonyl and sugar groups and the nitrogen atoms of the heterocyclic bases have a relatively low affinity for water molecules and play only a secondary role in the hydration of DNA. The ionic phosphate groups, on the contrary, invariably have a high affinity for water, and all evidence points to them as the primary hydration sites in DNA.

The hydration of DNA appears analogous to that of other ionic phosphate compounds, and can be regarded as the formation of a stable dihydrate at very low r.h., followed by the formation of a series of higher hydrates. Even small local differences may cause such hydrates to merge into one another.[9]

The Application of the BET Theory.—The adsorption data have been tested for a fit to the BET adsorption equation, introduced by Brunauer, Emmett and Teller,[10] and given a statistical mechanical foundation by Hill.[11] In Hill's notation, the BET equation is of the form

$$A = BCx/(1 - x + Cx)(1 - x) \qquad (1)$$

where in the present case A is the total amount of water adsorbed at relative humidity x, B is the maximum amount of water accommodated on the primary adsorption sites of DNA (first adsorbed layer) and C is a constant given by

$$C = \exp[(E_1 - E_L)/RT] \qquad (2)$$

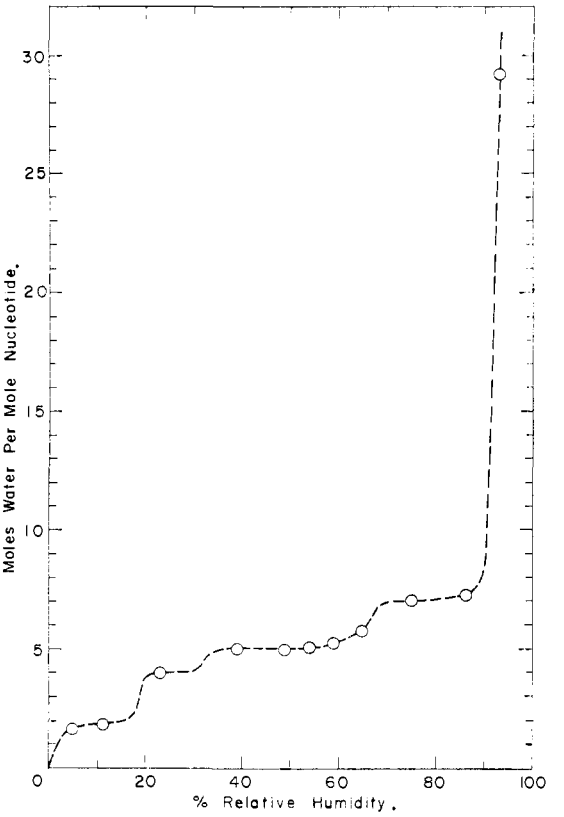

Fig. 2.—Adsorption of water by monosodium cytidine-5′-phosphate.

The constants E_1 and E_L are the adsorption energies for the first layer and for successive layers, respectively, the latter being further assumed equal to the energy of condensation of liquid water. Equation 2 omits a multiplicative constant,[12] which can be expressed in terms of partition functions of water in the first adsorbed layer and in succeeding layers.[13] The constant cannot easily be evaluated and setting it equal to unity, which was originally suggested by Brunauer, Emmett and Teller,[10] causes the calculated $(E_1 - E_L)$ values to lie generally below the experimental values, often by as much as 1 kcal./mole.[14,15]

The observed adsorption of water by NaDNA is compared in Table I with values calculated from eq. 1. The fit is very good up to 80% r.h. The best values of the BET parameters are $B = 2.2$ water molecules per nucleotide and $C = 21$ for NaDNA, while for LiDNA $B = 2.0$ and $C = 36$. The values of the constant B indicate that two molecules of water per nucleotide are adsorbed on the primary adsorption sites. From the values of the constant C and eq. 2 one calculates $(E_1 - E_L) = 1.7$ kcal./mole for NaDNA and $(E_1 - E_L) = 2.1$ kcal./mole for LiDNA. These two numbers are only rough estimates and may be low by as much as 1 kcal./mole, as pointed out above. Nevertheless the difference between LiDNA and NaDNA, 0.4 kcal./mole, is very likely real. The first two

(9) See, for example, G. D. Halsey in "Advances in Catalysis," Vol. 4, Academic Press, Inc., New York, N. Y., 1952, p. 259.

(10) S. Brunauer, P. H. Emmett and E. Teller, *J. Am. Chem. Soc.*, **60**, 309 (1938).

(11) T. L. Hill, *J. Chem. Phys.*, **14**, 263 (1946).

(12) S. J. Gregg and J. Jacobs, *Trans. Faraday Soc.*, **44**, 574 (1948).

(13) T. L. Hill, *J. Chem. Phys.*, **16**, 181 (1948).

(14) A. B. D. Cassie, *Trans. Faraday Soc.*, **41**, 450 (1945).

(15) R. A. Beebe, J. Biscoe, W. R. Smith and C. B. Wendell, *J. Am. Chem. Soc.*, **69**, 95 (1947).

water molecules are apparently bound more strongly to the $PO_2^-Li^+$ groups than to $PO_2^-Na^+$.

Applicability of the BET Model.—In view of the excellent fit of the data with the BET equation, the assumptions inherent in the BET model appear to be fulfilled for DNA. This is interpreted to mean that the adsorption of water by DNA occurs in "layers" around the phosphate groups, which are the primary adsorption sites. The first layer is held by strong adsorption forces, with a fairly sharp drop of about 2 kcal./mole in the adsorption energy between the first and the second adsorbed layers.

The data have also been tested for a fit with adsorption equations based on an extension of the BET model which allows a more gradual variation of adsorption energy from layer to layer. The first such equation, given in footnote 13 of the original BET paper,[10] allows the adsorbed water molecules to have values of adsorption energy E_1 and E_2 in first and second adsorbed layers different from E_L, which leads to an extra parameter, $C_2 = \exp[(E_2 - E_L)/RT]$. Further equations with more such parameters are easily deduced.

It was found that the only values of the parameters which would fit our data are very near to the simple BET results: B near 2.0, C_1 near 20, C_2 and C_3 near 1. This can be considered a confirmation that the two-parameter BET model adequately represents the hydration of DNA at low r.h. and that our physical interpretation of the BET constants is justified.

Deviations from the BET Equation.—Our data show a sudden negative departure from the BET equation above 80% r.h. for LiDNA and for NaDNA. Of several possible causes of such a deviation[16] the most likely is the filling of the space available to the incoming water molecules. After the "empty space" between DNA molecules is filled, further hydration must push the DNA molecules apart. This expansion requires energy and could account for a negative deviation from the BET model. It is in fact found that the swelling of DNA, as estimated from the increase in the equatorial X-ray diffraction spacings,[5,17] begins to increase very sharply between 80 and 85% r.h.

The adsorption–desorption hysteresis is probably also connected with the filling of the "empty space" and we should therefore predict that it ought to be limited to the r.h. range where such space is being filled. Since hysteresis occurs in the range from 10% to 80% r.h., it appears that the DNA double helices become completely surrounded by water at 80% r.h. or a little below this point. At 80% r.h. about 36% of the total weight of DNA is due to water, hence (assuming unit density of water and 1.5 as the density of dry DNA[5]) roughly 46% of the total volume of the hydrated DNA is water.

Acknowledgment.—We wish to thank Dr. Peter F. Davison for advice regarding the sodium–lithium exchange and for some specially prepared samples of NaDNA, and Prof. Alexander Rich for comments on the manuscript and for helpful discussions.

(16) W. G. McMillan and E. Teller, *J. Chem. Phys.*, **19**, 25 (1951).
(17) A. C. T. North and A. Rich, *Nature*, **191**, 1242 (1961).