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THE NET HYDRATION OF DEOXYRIBONUCLEIC ACID,†*

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In 1954, Jacobson *et al.*[1] presented evidence for the extensive hydration of DNA from studies of the proton magnetic resonance in aqueous solutions of sodium DNA. The following year Wang[2] concluded that DNA was hydrated to the extent of only 0.35 gm water/gm dry deoxynucleate from self-diffusion measurements of water in NaDNA solutions. This paper presents evidence for a net hydration of 0.2 to 2 gm water/gm CsDNA in certain buoyant solvents.

The hydration of T-4 bacteriophage DNA[3] has been studied in density gradient systems at sedimentation equilibrium in the ultracentrifuge.[5] Williams *et al.*[6] showed that the buoyant density is that of the solvated species. The buoyant density ρ_0 is defined by the following thermodynamic equations:

$$\frac{1}{\rho_0} = \frac{M_3\bar{v}_3 + \Gamma M_1\bar{v}_1}{M_3 + \Gamma M_1},$$

where 1 refers to water, 3 to the unhydrated polymer, and

$$\Gamma = -\left(\frac{\partial\mu_1}{\partial m_3}\right)_{T,P,m_1} \Big/ \left(\frac{\partial\mu_1}{\partial m_1}\right)_{T,P,m_3} = \left(\frac{\partial m_1}{\partial m_3}\right)_{T,P,\mu_1}.$$

M, $\bar{v}$, μ, and m are molecular weights, partial specific volumes, chemical potentials, and molalities respectively. Molalities for this equation are expressed in moles per unit weight of salt. The unusual definition of molality is necessary so that Γ, the net solvation, remains a positive quantity.[7]

The net solvation of DNA is shown here to be a monotonic function of water

activity. Introducing the concept of water activity into the problem of polymer hydration has proven useful.

The first data were obtained on solution mixtures of CsBr and LiBr. On addition of small amounts of LiBr to CsBr solution there is a sudden drop in the buoyant density associated with the replacement of Cs ions on the DNA by Li ions (Fig. 1).

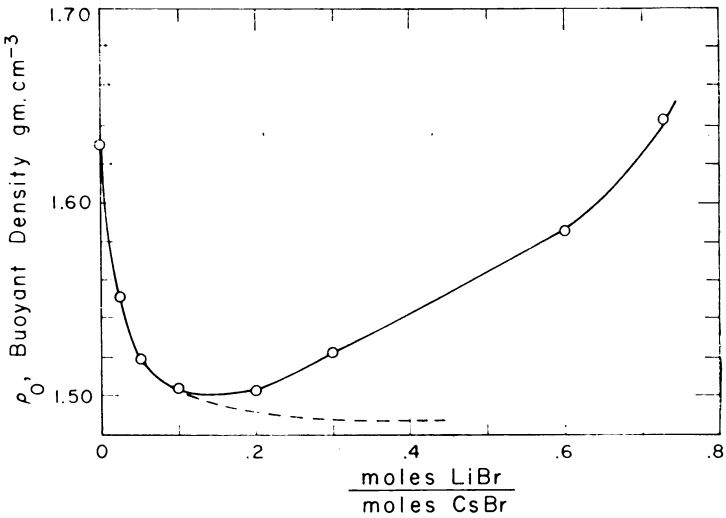

FIG. 1.—Buoyant density of T-4 bacteriophage DNA in aqueous mixtures of LiBr and CsBr at 25°C.

In the event that solvation of the DNA does not change, it can be shown that the plot of ρ_0 against the mole ratio Li/Cs in the solution should be approximately hyperbolic. The limiting value of ρ_0 at Li/Cs = ∞ is the buoyant density of the solvated lithium DNA. Curves of this form have been obtained for mixtures of CsBr and guanidinium bromide and for CsCl and guanidinium chloride.[8]

Although the curve for LiBr-CsBr mixtures is hyperbolic at low LiBr concentration, the buoyant density increases at high LiBr concentrations. At these LiBr concentrations the DNA is entirely in the lithium form as indicated in Figure 1 by the dashed hyperbola. The increase in buoyant density of the solvated LiDNA is then to be expected, as solvation should decrease at the low-water activities in concentrated lithium bromide solutions.

The same data with additional points have been plotted against water activity in Figure 2. The water activities were calculated with the Guggenheim rule for mixed electrolytes and with data for osmotic coefficients tabulated by Robinson and Stokes.[9] Because osmotic coefficients for CsBr are not available at high mixed salt molalities, the osmotic coefficients of CsCl were used for CsBr for total molalities between 5 and 10. Above molality 10 the osmotic coefficient of CsBr was taken to be 1.02. Errors resulting from this procedure are small as the mole fraction of CsBr is small. The buoyant density in aqueous LiBr was not obtained because the salt is not soluble enough at 25°C.

A second method demonstrating the effects of water activity on buoyant density is shown in Figure 3. The buoyant densities[10] of T-4 DNA in different cesium

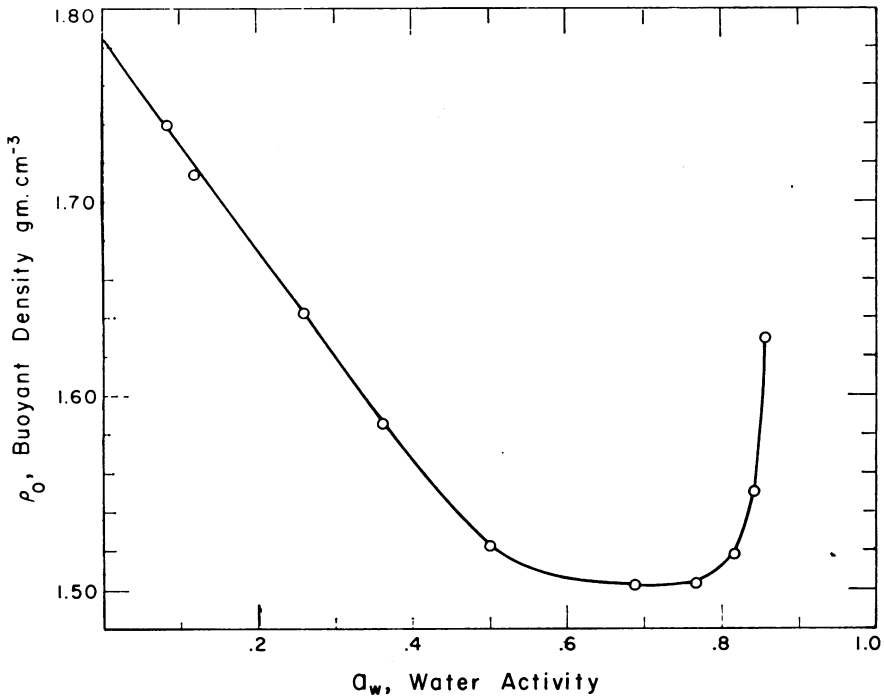

FIG. 2.—Buoyant density of T-4 bacteriophage DNA in aqueous mixtures of LiBr and CsBr at 25°C.

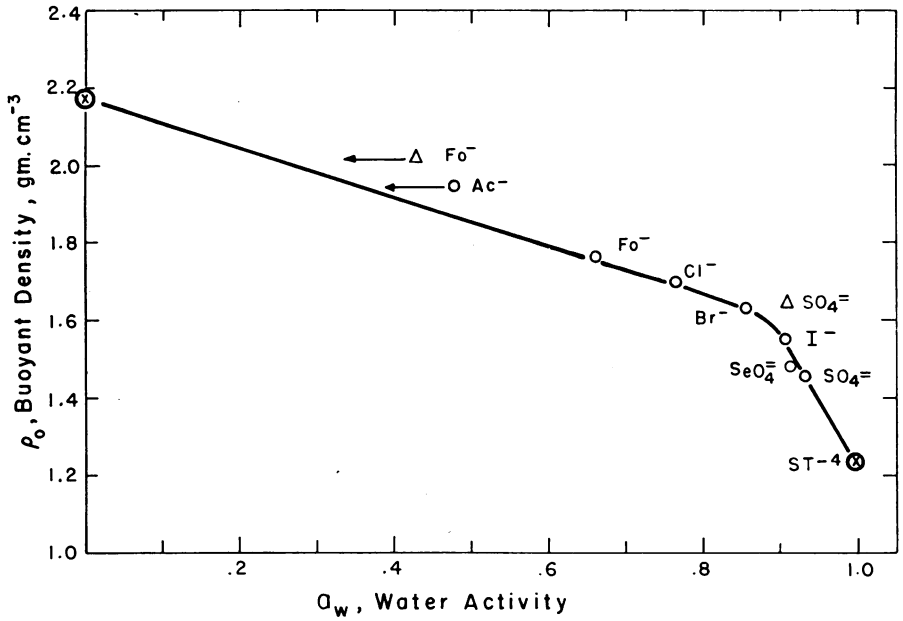

FIG. 3.—Buoyant density in various aqueous cesium salt solutions. T-4 bacteriophage DNA, O; Pea seedling ribosomal RNA, Δ, in $SO_4^=$; *Escherichia coli* ribosomal RNA, Δ in Fo^-. The extreme DNA values, ⊗, were obtained with LiDNA and recalculated for CsDNA as described in the text. Fo^-, Ac^-, St^{-4} refer to the formate, acetate, and silicotungstate ions.

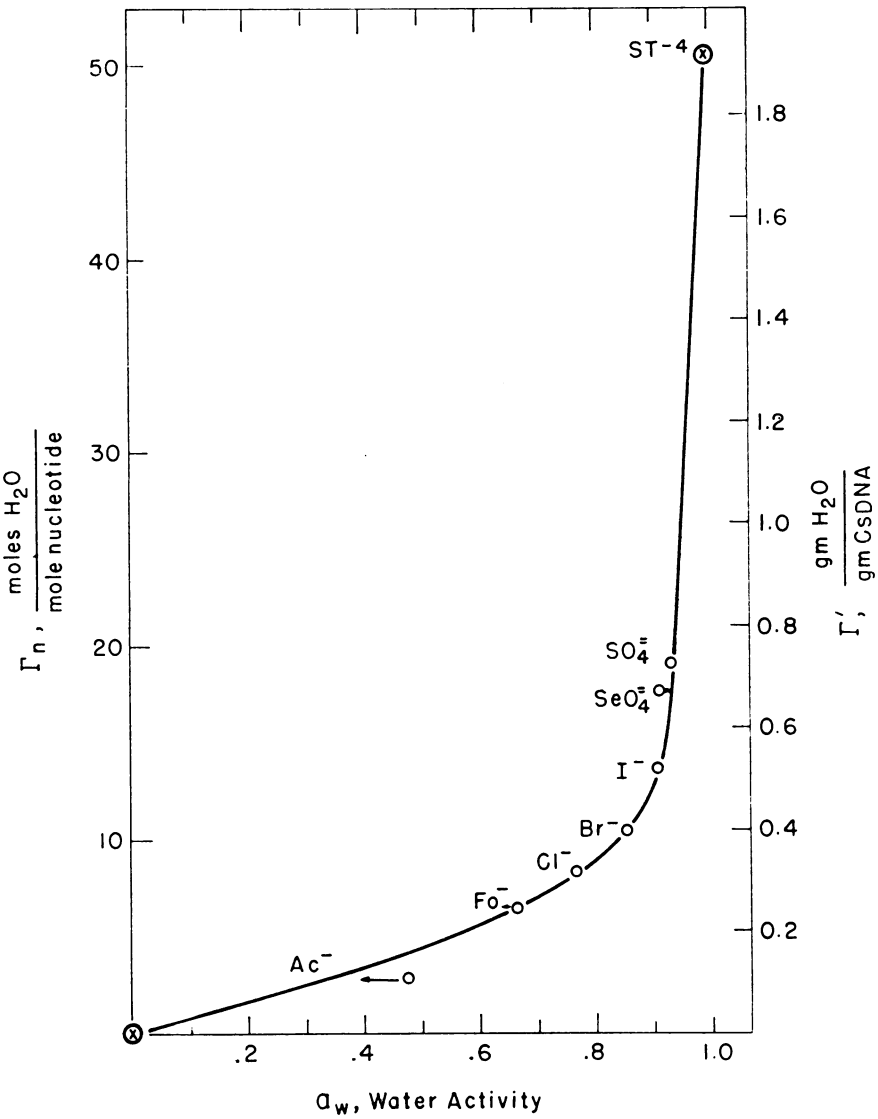

FIG. 4.—The net hydration for T-4 bacteriophage CsDNA at 25°C.

salt solutions are plotted against water activity, again calculated from data in Robinson and Stokes.[9] Osmotic coefficients for points shown with arrows are not available at present. For these, water activities were calculated assuming an osmotic coefficient of one. The direction of the arrow indicates the way the point is expected to move when current isopiestic measurements on these solutions are completed. The data in this paper will be presented with the corrected activity data in a later publication together with the theory of solvation in the density gradient system.

The extrapolated value of CsDNA (Fig. 3) at water activity a_w equal to zero was calculated from the linearly extrapolated LiDNA value (Fig. 2) using an average

nucleotide ion residue weight of 340.[11] The specific volume of the DNA ion was calculated from the extrapolated LiDNA buoyant density assuming the lithium ion added weight but no volume to the DNA. The maximum error resulting from this assumption is 2 per cent of the specific volume. The buoyant density for CsDNA at $a_w = 0$ was then calculated using the difference in the molar volumes of the Cs ion and the Li ion obtained from the difference in the crystal molar volumes of CsBr and LiBr. In these concentrated salt solutions, partial molar volumes and crystal molar volumes are almost identical; consequently little error results from this procedure. The value obtained in this calculation was 2.117 ± .040 gm/cc. It should be pointed out that this number is the reciprocal of the specific volume of unhydrated CsDNA. We have learned[13] that the reciprocal of the partial specific volume of CsDNA in water is 2.24 gm/cc. Comparison of the two numbers yields a valuable method of determining the density of the hydrated water at $a_w = 1$ and suggests that the hydrated water has nearly the same density as the water in the solution.

Figure 3 also shows 2 RNA points. The Cs formate point was taken from Davern.[14] The Cs_2SO_4 point was measured by the authors at pH 5.5.[15] These points suggest that there is a small difference in the solvation of RNA and DNA,[16] but the data are too incomplete to make a definite statement. The extrapolated value for anhydrous CsRNA is expected to be less than 0.1 density units greater than the value for CsDNA.

LiDNA has been banded in lithium silicotungstate at pH 4.7. The buoyant density of lithium DNA in this solution was 1.138 gm/cc. The water activity calculated for this solution assuming an osmotic coefficient of one is 0.995. The amount of water per nucleotide was calculated from the extrapolated LiDNA density at $a_w = 0$. The buoyant density was then corrected to CsDNA (Fig. 3) assuming that CsDNA and lithium DNA are equally solvated.

Figure 4 shows Γ_n against a_w. Γ_n is defined as moles water per mole average nucleotide and was calculated[17] for an average nucleotide ion residue weight of 340, assuming the density of the solvated water to be 1.00 gm/cc.

Hydration and dehydration have long been suspected of having an important role in the control of cellular replication.[18] The rapid change in hydration of DNA between $a_w = 0.9$ and $a_w = 1.0$ indicates that such mechanisms should be seriously examined.

Our sincere thanks are extended to F. Bonhoeffer and H. Schachman for permitting us to publish the partial specific volume of CsDNA. We wish also to thank Dr. P. O. P. T'so[19] for the RNA sample.

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‡ Predoctoral Fellow of the National Science Foundation.

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[7] The two equations are essentially those of Williams *et al.*,[1] equations (77) and (35), except for a change in the choice of the dependent variables.

[8] From the hyperbola it is possible to calculate a relative binding constant for the two ions on the DNA.

[9] Robinson, R. A., and R. H. Stokes, *Electrolyte Solutions* (New York: Academic Press, 1955), pp. 33, 426, 468–489.

[10] The CsI point is only approximate because the buoyant density was only slightly less than that of a saturated CsI solution at about 200 atmospheres pressure at 25°C. Emission spectrographic analysis of all the cesium salts indicates high cation purity.

[11] This average nucleotide ion residue weight was calculated from the base compositions for T-4 DNA given in Adams,[12] taking into account the glucose on the hydroxymethylcytosine.

[12] Adams, M. H., *Bacteriophages* (New York: Interscience Publishers, Inc., 1959), pp. 91–92.

[13] Private communication, F. Bonhoeffer and H. Schachman.

[14] Davern, C., Doctoral Dissertation, California Institute of Technology, p. 108 (1959).

[15] The RNA was supplied by P. T'so and prepared as described in his publication.[19] The sample was pea seedling microsomal RNA.

[16] An explanation for at least part of the large differences in buoyant density of CsDNA and CsRNA in CsFormate and CsCl solutions is the fact that the water activity of each of these solutions is such a strong function of solution density.

[17] Γ_n was calculated with the equation

$$\frac{1}{\rho_0} = \frac{v_{DNA} + \Gamma'_{H_2O}}{1 + \Gamma'}$$

where Γ is the solvation expressed on a weight basis. The specific volume of the solvated water, v_{H_2O}, is taken to be one, and v_{DNA} is the extrapolated specific volume of CsDNA at $a_w = 0$.

[18] Serra, J. A., *Encyclopedia of Plant Physiology* (Berlin: Springer-Verlag, 1955), pp. 472–499.

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*TYPES AND FREQUENCIES OF HUMAN CHROMOSOME ABERRATIONS INDUCED BY X-RAYS**

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In spite of widespread concern over the genetic hazards to human populations of ionizing radiations, relatively few quantitative data based on direct determinations of the genetic effects of radiations on human beings are as yet available. The difficulties inherent in genetic studies with individuals or populations exposed to ionizing radiations is evident in the reports of Neel and Schull[1] dealing with the Japanese populations exposed at Hiroshima and Nagasaki. Hence, alternative approaches to this problem seem highly desirable. One such approach is now possible as a result of recent advances in mammalian tissue and cell culture techniques. These advances make possible the initiation, prolonged maintenance with active growth, and effective chromosomal study of normal euploid human cell cultures. Thus, these developments have made available experimental material suitable for studies of radiation-induced aberrations in human chromosomes.