THE SEDIMENTATION OF DEOXYRIBONUCLEIC ACID IN THREE COMPONENT SYSTEMS*

by

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INTRODUCTION

The sedimentation of proteins and viruses in solvent systems of different densities has been used for many years[1] in attempts to measure the density or hydration of these macromolecules. These experiments consist of the determination of the sedimentation velocities of the macromolecules in buffered solutions to which increasing amounts of some additional solute, such as glycerol, sucrose or serum albumin, have been added in order to increase the density of the medium. From the dependence of the sedimentation coefficient on the density of the medium a particular density, ϱ^0, can be found, often by extrapolation of the experimental data, at which the sedimentation rate of the particles would be equal to zero. This density is then taken to be the density of the particles.

Many different solutes have been used to increase the density of the medium and it is implicit in the method that there should be no interaction between the sedimenting macromolecule and the solute which is used to increase the density. Even for those systems in which it is likely that there is only weak Van der Waals attraction between the macromolecule and the added solute it is found that different values of ϱ^0 obtain depending on the solute which is used to increase the density. Thus bushy stunt virus appears to be less dense in sucrose solutions than in glycerol solutions[2]. In more complicated systems, tobacco mosaic virus (TMV)[3] and southern bean mosaic virus[4] appear to be less dense in serum albumin solutions than in sucrose solutions.

Several years ago, KAUZMANN[3] suggested a simple explanation for these results. He pointed out, in effect, that in these multicomponent systems the solvent containing sucrose or serum albumin molecules could not be considered a continuum with regard, to the sedimenting particles as is customarily assumed for a two component system. According to his physical picture, there will be steric exclusion of sucrose or serum albumin molecules from a region immediately surrounding the sedimenting particles, and the extent of this region will be a function of the size of the molecules added to increase the density of the medium. In this way, these seemingly anomalous results could be explained.

In this communication we are reporting the results of experiments on the sedimentation of deoxyribonucleic acid (DNA) in buffered solutions containing varying

* These studies have been aided by a contract between the Office of Naval Research, Department of the Navy, and the University of California NR 121–175, and also by grants from Lederle Laboratories and the Rockefeller Foundation.

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amounts of D_2O in one series of runs and differing concentrations of sucrose in another series of runs. Because of its size, shape and sedimentation behavior[5,6], DNA offers special advantages as a model for further investigations of sedimentation in three component systems. The results have been interpreted with the ideas proposed by KAUZMANN and the theory of GOLDBERG[7] for sedimentation in multicomponent systems. The equations resulting from this thermodynamic treatment are similar in many respects to some derived earlier by another method[8,9], and they show clearly that these sedimentation experiments measure only the preferential binding of the macromolecule for one of the components of the solvent.

THEORY

The theory of the sedimentation velocity of macromolecules in multicomponent systems has been independently discussed by several authors[7,8,9], and the treatment which follows is an explicit application of GOLDBERG's general formulation[7] for three component systems.

In each of the systems DNA–H_2O–D_2O–buffer and DNA–H_2O–sucrose–buffer, the concentrations of DNA and buffer were maintained constant from experiment to experiment while the D_2O and sucrose concentrations were varied in order to produce solutions of different density. Throughout the discussion the subscript 1 refers to H_2O, 2 corresponds to *dry* DNA and 3 refers either to sucrose or to D_2O as the context indicates.

For such systems, at great dilution of DNA we may write[7]

$$f'\eta s = M\,(1 - \bar{V}\varrho) \tag{1}$$

where η and ϱ are the viscosity and density of the medium, f' is a function of the shape and size of the sedimenting unit, and s, M, and $\bar{V}$ are, respectively, the sedimentation coefficient, molecular weight and partial specific volume of the *sedimenting unit.*

The composition of the sedimenting unit will, in general, be unknown since the macromolecule may have associated with it water, sucrose and buffer, or water, D_2O and buffer. In the treatment which follows we will ignore the contribution of the buffer and consider the sedimenting unit as a particle consisting of DNA plus whatever other components of the solution are associated with it as a kinetic unit*.

We can write that $(kn_1 + w)$ molecules of water and kn_3 molecules of sucrose (or D_2O) are bound to a single molecule of DNA without specifying the nature of the forces involved. Here n_1 is the total number of molecules of water in the centrifuge cell, n_3 is the total number of sucrose (or D_2O) molecules, k is a proportionality constant and w we shall designate the preferential adsorption coefficient for water. If we permit k to have any value, $k \geq 0$, and w to be positive, negative or zero then the above description of the amounts of water and sucrose (or D_2O) bound by DNA covers all possibilities. In particular, if $w = 0$ and $k = 0$, the sedimenting unit contains no components of the solvent; if $w = 0$ and $k > 0$, the associated solvent has the same composition as the bulk-mixed solvent; and finally if $k > 0$ and $w \neq 0$ there will be preferential binding of either water ($w > 0$) or sucrose ($w < 0$).

* The words "associated" or "bound" must not be taken to imply rigid binding of solvent. Although such binding may exist, the experiments described here are incapable of distinguishing between this kind of binding and much looser kinds of association.

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If we consider that a molecule of DNA reacts with water and sucrose (or D_2O) as indicated above, then from thermodynamics we can write for the chemical potential, μ, of the sedimenting unit

$$\mu = \mu_2 + (kn_1 + w)\mu_1 + kn_3\mu_3. \tag{2}$$

Differentiation of this equation with respect to pressure, assuming that coefficients of the μ_i are independent of pressure, leads directly to the partial specific volume of the sedimenting unit.

$$\bar{V} = \frac{M_2\bar{V}_2 + (kn_1 + w)M_1\bar{V}_1 + kn_3M_3\bar{V}_3}{M_2 + (kn_1 + w)M_1 + kn_3M_3}. \tag{3}$$

Combinations of equations (3) and (1) leads to

$$f'\eta s = M_2(1 - \bar{V}_2\varrho) + wM_1(1 - \bar{V}_1\varrho) + kn_1M_1(1 - \bar{V}_1\varrho) + kn_3M_3(1 - \bar{V}_3\varrho). \tag{4}$$

The last two terms on the right hand side of equation 4 are equal to $Nk(g_1 + g_3) - Nk\varrho(g_1V_1 + g_3V_3)$ where g_i is the total number of grams of i in the centrifuge cell and N is Avogadro's number. At infinite dilution of DNA the sum of these two terms is zero and equation 4 reduces to

$$f'\eta s = M_2(1 - \bar{V}_2\varrho) + wM_1(1 - \bar{V}_1\varrho) \tag{5a}$$

and

$$w^0 = -M_2(1 - \bar{V}_2^0\varrho^0)/M_1(1 - \bar{V}_1^0\varrho^0) \tag{5b}$$

where the superscript, zero, denotes the values at $s = 0$.

This derivation shows that kn_1 and kn_3, no matter how large, can have no effect at all on the product $f'\eta s$. It is true that the friction factor, f', is dependent on the amount of liquid associated with a DNA molecule but the sedimentation coefficient is affected also and in such a way that $f'\eta s$ remains constant. Only water (or sucrose) bound in excess of the ratio in which they exist in the bulk solution affects the product $f'\eta s$ and only this water (or sucrose) can be determined in unambiguous fashion by the type of experiments reported here.

In consideration of the experiments in which D_2O is used to increase the density of the solution, we can make the additional assumption that there will be no difference in binding of either H_2O or D_2O to the macromolecule, *i.e.* $w = 0$ for the experiments with D_2O. Equation 5a then reduces to

$$f'\eta s = M_2(1 - \bar{V}_2\varrho). \tag{6}$$

Equation 6 provides the theoretical justification for the determination of partial specific volumes by sedimentation experiments in H_2O–D_2O mixtures[2,10,11,12].

Equations 5 and 6 are adequate for the interpretation of previous experiments and those described in this communication. If it is permissible to ignore the buffer, then equation 5 is, moreover, exact while equation 6 is limited only by the postulated equivalence of H_2O and D_2O. Of course, there may be some exchange of deuterium for hydrogen in the macromolecule[13] but this should effect only a very slight change in M_2 or $\bar{V}_2$. It should be noted that these equations are very similar to those derived by entirely different methods[8,9].

The development of the theory as presented here, however, gives no indication of the forces responsible for the origin of w. KAUZMANN has pointed out one of the factors

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which will lead to a value of w greater than zero and it is of interest to see whether the effect which he described can account for the experimental results or whether we must invoke other factors.

In this view sucrose is taken to be an incompressible sphere of radius a. The center of the sucrose molecule can, accordingly, approach the "surface" of a DNA molecule no closer than the distance a. This results in a region of thickness $2a$ surrounding the macromolecule in which the density varies from that of water to that of the sucrose solution. According to KAUZMANN this region of varying density can be approximated by assigning a water layer of thickness a to the macromolecule and assuming that the liquid more distant than a from the surface of the macromolecule has the density of the bulk mixed solvent. If this shell of water has a volume, Δv, then we can write

$$w_s = N\varrho_1 \Delta v / M_1 \tag{7}$$

where w_s is the preferential adsorption coefficient due to steric exclusion at the surface and the other terms have the meaning assigned earlier. If we assume that the DNA molecule may be represented by a long solid cylinder, Δv would equal $\pi h(R^2 - r^2)$ where R is the radius of the sedimenting unit, *i.e.* containing the shell of water which is preferentially associated, r is the radius of the dry DNA molecule and its non-preferentially adsorbed solvate (if any), and h is the contour length of the cylinder. Equation 7 can now be written

$$w_s = N\varrho_1 \pi h(R^2 - r^2)/M_1. \tag{7b}$$

It should be emphasized that there may be water or sucrose or both within the DNA molecule and that equation 7b can yield no information regarding preferential adsorption of water within the macromolecule or even at the surface of the macromolecule, except for that water preferentially associated with the macromolecule due to steric exclusion of the other component of the mixed solvent.

METHODS

DNA was prepared from calf thymus by the method of SIGNER AND SCHWANDER[14] with only slight modifications in manipulation. It has the fibrous appearance typical of high molecular weight preparations of DNA precipitated from ethanol. A stock solution of known concentration of DNA in distilled water was prepared from a partially dried sample whose water content had been previously obtained by drying a portion of the sample to constant weight in a vacuum oven, initially at 85° C and finally at 106° C in vacuum. No significant differences were observed between the dry weights obtained at the two temperatures.

To prepare the DNA-H_2O-sucrose-buffer systems, aliquots of the DNA stock solution, identical in weight to within 0.1%, were weighed into a series of volumetric flasks, followed by the addition and weighing of increasing amounts of dry sucrose. A concentrated citrate buffer solution and then distilled water were added so that the final solutions were 0.6 ionic strength at pH 6.9 and the concentration of DNA was 0.03%. The care taken in reproducing the concentration of DNA from sample to sample was necessitated by the fact that the sedimentation coefficient of DNA is markedly dependent on concentration[6,15]. At the concentration used in this study, however, there is only a slight increase in sedimentation coefficient during a single ultracentrifuge run as the concentration of the sedimenting material is decreased owing to the shape of the ultracentrifuge cell and the radial inhomogeneity of the centrifugal field[16].

The solutions containing D_2O were prepared in a similar manner except that the D_2O was delivered volumetrically.

For each ultracentrifuge experiment the viscosity and density of another sample, identical in composition except for the absence of DNA, were measured at 25° C in an Ostwald type viscometer and a pyknometer of 2 ml volume.

A Spinco Model E ultracentrifuge equipped with a schlieren optical system was used for the

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centrifugation studies. All runs were made at 59,780 r.p.m. in the conventional rotor and all but two were in the 12 mm cells supplied with the instrument. The two exceptions were the runs at the two highest sucrose concentrations which were made in a synthetic boundary ultracentrifuge cell. At these high concentrations of sucrose the observed sedimentation of the DNA is so small (because of the high viscosity and density of the solutions) that appreciable redistribution of the sucrose occurs before the DNA boundary has migrated a sufficient distance from the meniscus to allow precise measurements of the boundary positions to be made. Furthermore, the variation of viscosity of sucrose solutions with concentration is very great in this concentration region, so that the viscosity of the solution in the boundary region in a conventional cell would be much less than the macroscopic viscosity measured before the run began. This effect can be avoided by forming the boundary near the center of the ultracentrifuge cell where the concentration of the sucrose solutions is almost independent of the time of centrifugation. The measured viscosity and density of the solvent can then be considered equal to the viscosity and density prevailing during the ultracentrifuge measurements. At the lower concentrations of sucrose, the viscosity is less dependent on the concentration of sucrose and the ultracentrifuge boundary moves sufficiently rapidly that the effect of redistribution of the sucrose may be neglected.

The photographic plates were read with a microcomparator and the sedimentation coefficients at the average temperature of the run were obtained from the initial slopes of plots of log x *vs.* t where x is the distance of the boundary from the axis of rotation and t is the time in seconds. A slight upward curvature of these plots was evident in some runs characteristic of the dilution of the DNA during the run, but measurement of the slopes at zero time yielded the sedimentation coefficient at the initial concentration of DNA which was the same in all runs.

RESULTS AND DISCUSSION

Fig. 1 shows the results of the sedimentation studies of DNA, in D_2O and sucrose solutions. The straight lines were calculated by the method of least squares and the values of ϱ^0 were obtained from the equations of these lines. For the experiments in D_2O solutions, ϱ^0, by extrapolation, was 1.68 g/ml and for the experiments in sucrose solution it was 1.42 g/ml. The reciprocal of these values, 0.59 ml/g and and 0.70 ml/g, are equal in the first instance to the partial specific volume of *dry* DNA in a D_2O–H_2O solution of density 1.68 g/ml and in the second instance to the partial specific volume of the sedimenting unit in a sucrose solution of density 1.42 g/ml.

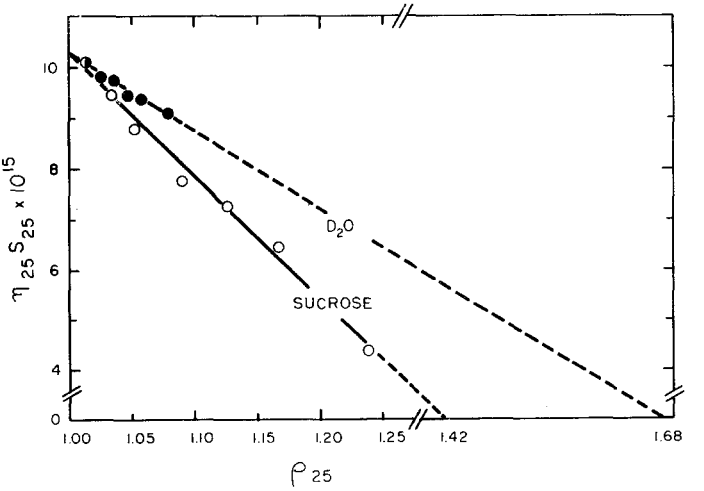

Fig. 1

Measurements of the apparent specific volume of DNA have yielded values ranging from 0.47 to 0.66 ml/g[17]. The currently accepted value for the partial specific volume

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is 0.55 ml/g[15]. In view of the long extrapolation required because of the low partial specific volume of DNA, our value of about 0.59 ml/g, obtained from sedimentation experiments in D_2O–H_2O mixtures is in satisfactory agreement with the best data in the literature.

The method of preparation of the DNA is essentially identical with that used previously by one of us[18], and we may therefore take $5.8 \cdot 10^6$ as the molecular weight of DNA[18,19]. Assuming that the partial specific volume of DNA in the sucrose solutions is also 0.59 ml/g we calculate w^0 to be $1.2 \cdot 10^5$ from Equation 5b. It is much more difficult to obtain a reliable value of w_s for comparison with w^0 because of the theoretical and experimental difficulties involved in determining h and r. From data obtained from electron microscopy[20,21], sedimentation[6], light scattering[19] and X-ray diffraction[5,22,23] measurements we take $r = 10$ A and $h = 20{,}000$ A as the most probable values. With the assumed value, 5 A, for the "radius" of a sucrose molecule we calculate the value, $2.6 \cdot 10^5$, for w_s.

For comparison we can make similar calculations from the sedimentation data of tobacco mosaic virus in sucrose solutions[3], and we find $w^0 = 6.0 \cdot 10^5$ and $w_s = 2.3 \cdot 10^5$. In the case of tobacco mosaic virus where $w^0 > w_s$, it was previously suggested on the basis of a slightly different calculation, that the virus particles possess a real hydration layer. No such explanation avails for the DNA experiments, however, since here $w^0 < w_s$. A reasonable explanation for this result arises from a reconsideration of the model used for DNA in the calculation of w_s. We have assumed the DNA molecule to be a solid cylinder of about 20 A diameter, but examination of the two strand, helical model of WATSON AND CRICK[5] suggests that there are regions between the successive turns of the helix which are accessible to sucrose. Such a partial permeation of the DNA molecule by sucrose would result in a smaller effective thickness of the DNA molecule which would lead to a smaller calculated value for w_s.

The data on DNA and also those obtained earlier on tobacco mosaic virus can not be used as a rigorous test of the validity of the KAUZMANN suggestion despite the fact that this idea provides a reasonable explanation for the behavior observed for these two materials. Until we have a model for which the steric effect is reasonably large and for which we know that w arises solely from steric exclusion at the surface, we will not be able to eliminate the uncertainties arising either from internal selective absorption of water or partial permeation of the macromolecule by a third component.

Since these experiments measure only the water preferentially associated with the macromolecule and not the total amount of water, they can be conside redas a means for determining the minimum amount of water associated with a macromolecule. For the purpose of describing the experiments in sucrose, we could equally well have assumed that there was preferential adsorption of sucrose to the macromolecule. In such a case the term $wM_1(1 - \bar{V}_1\varrho)$ in equation 5 would be replaced by a term $sM_3(1 - V_3\varrho)$ where s is the preferential adsorption coefficient for sucrose. We would have found $s < 0$, however, which indicates that $w > 0$,and hence we preferred the method used. The importance of s lies in the fact that WALES AND WILLIAMS[9] have explicitly identified it with $(\delta m_3/\delta m_2)_{T,P,\mu_3}$ used by STOCKMAYER[24] in his formulation of the theory of light scattering in multicomponent systems. It has also been shown that this quantity is the number of moles of component 3 bound preferentially by one mole of the macromolecule and it can be determined, in principle, by equilibrium dialysis measurements[25]. It should be noted that in addition to the electrostatic and Van der Waals forces commonly used

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to interpret the results of equilibrium dialysis experiments consideration must be given to steric effects such as those considered for ultracentrifugation. Thus there can be apparent binding between a macromolecule and one of the components of the solvent even though there is no attractive potential between that component and the macromolecule. Similar considerations also apply to light scattering studies of three component systems.

It should be noted that equation 5 provides a basis for the extension of the theory of SCHERAGA AND MANDELKERN[26] to three component systems. As the authors pointed out, their equation 6 is only approximately true for three component systems. Using our equation 5 we can write for β, defined by them

$$\beta = \frac{NsM_2^{1/3}[\eta]^{1/3}\eta_1}{M_2(1 - \bar{V}_2\varrho) + wM_1(1 - \bar{V}_1\varrho)} \tag{8}$$

For experiments such as those described above where $w > 0$ and $(1 - \bar{V}_1\varrho) < 0$ neglect of the second term in the denominator of equation 8 yields a value of β which is too small. This will lead to an erroneous value for the axial ratio of the equivalent hydrodynamic ellipsoid. For those systems in which only weak Van der Waals forces exist between component three and the macromolecule, KAUZMANN's theory should serve as a useful guide in estimating whether w may be neglected.

SUMMARY

The sedimentation of deoxyribonucleic acid (DNA) was studied in three component systems containing DNA, D_2O and buffer in one series of experiments and DNA, sucrose and buffer in another series. By varying the concentration of D_2O and sucrose in these experiments it was possible to measure the dependence of sedimentation coefficient of DNA on the density of the medium. Extrapolation of the data gave the values, 1.68 g/ml and 1.42 g/ml, for the density of D_2O and sucrose solutions, respectively, in which DNA has zero sedimentation rate.

A theoretical treatment based on GOLDBERG's general thermodynamic formulation of sedimentation in multicomponent systems is presented. Though the formulation is different from that of other authors, the final equations bear a marked similarity to those derived earlier. It is shown that the experiments in D_2O yield the partial specific volume of DNA and the value, 0.59 ml/g, obtained in this way is in satisfactory agreement with the presently accepted value. The experiments in sucrose solutions give the preferential adsorption of water by DNA and the value obtained is discussed with reference to KAUZMANN's proposal of steric exclusion of solute molecules from the surface of a macromolecule. The value calculated from KAUZMANN's proposal is larger than that observed experimentally, and the discrepancy is interpreted in terms of the WATSON-CRICK model for DNA as indicating that the macromolecule has an open structure which allows penetration not only of water but also of sucrose to a smaller extent.

Finally the interrelationship between this type of experiment in multicomponent systems to light scattering and equilibrium dialysis measurements is discussed.

RÉSUMÉ

La sédimentation de l'acide désoxyribonucléique (DNA) a été étudiée dans des systèmes à trois constituants renfermant DNA, D_2O et tampon dans une première série d'expériences et DNA, sucrose et tampon dans une deuxième. En faisant varier les concentrations en D_2O et en sucrose au cours de ces expériences, les auteurs ont pu mesurer l'influence de la densité du milieu sur le coefficient de sédimentation du DNA. L'extrapolation des résultats fournit, pour les densités des solutions de D_2O et de sucrose, dans lesquelles le DNA a une vitesse de sédimentation nulle, les valeurs de 1.68 g/ml et 1.42 g/ml, respectivement.

Un traitement théorique fondé sur la formulation thermodynamique générale, due à GOLDBERG, de la sédimentation dans les systèmes à plusieurs constituants est présenté. Quoique la formulation diffère de celle fournie par d'autres auteurs, les équations finales ressemblent nettement à celles établies précédemment. Les auteurs montrent que les expériences dans D_2O donnent le volume partiel spécifique du DNA et la valeur, 0.59 ml/g, obtenue de cette façon est en bon accord avec la valeur admise actuellement. Les expériences dans les solutions de sucrose fournissent l'adsorption

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préférentielle de l'eau par le DNA et la valeur obtenue est discutée par rapport à l'hypothèse de KAUZMANN sur l'exclusion stérique des molécules de soluté de la surface d'une macromolécule. La valeur calculée à partir de l'hypothèse de KAUZMANN est plus grande que celle observée expérimentalement et ce désaccord est interprété à l'aide du modèle de DNA de WATSON-CRICK comme indiquant que la macromolécule a une structure ouverte qui permet la pénétration non seulement de l'eau mais aussi, dans une plus faible mesure, du sucrose.

Enfin les relations entre ce type d'expériences dans des systèmes à plusieurs constituants et les déterminations fondées sur la dispersion de la lumière et la dialyse à l'équilibre sont discutées.

ZUSAMMENFASSUNG

Die Sedimentierung von Desoxyribonukleinsäure (DNA) wurde bei aus drei Komponenten bestehenden Systemen untersucht, welche in der einen Versuchsserie DNA, D_2O und Puffer, in der anderen jedoch D_2O, Saccharose und Puffer enthielten. Durch Variation der D_2O- und Saccharosekonzentration im Laufe dieser Versuche, konnten die Abhängigkeit des DNA-Sedimentierungskoeffizienten von der Dichte des Mediums gemessen werden. Extrapolation der Ergebnisse bis DNA-Sedimentierungsgeschwindigkeit gleich Null ergab die Werte 1.68 g/ml und 1.42 g/ml für die Dichte der D_2O- bezw. Saccharoselösungen.

Auf Grund von GOLDBERGS allgemeiner thermodynamischer Formulierung der Sedimentierung in multikomponenten Systemen, werden die Ergebnisse theoretisch behandelt. Obgleich sich die Formulierung von denjenigen anderer Autoren unterscheidet, weisen die Endgleichungen eine bedeutende Ähnlichkeit mit den früher erhaltenen Gleichungen auf. Es wird bewiesen, dass die Versuche in D_2O das partielle spezifische Volumen von DNA ergeben; der so erhaltene Wert von 0.59 ml/g stimmt zufriedenstellend mit dem gegenwärtig angenommenen Werte überein. Die in Saccharoselösungen durchgeführten Versuche ergeben die preferentielle Adsorption von Wasser durch DNA; der erhaltene Wert wird auf Grund von KAUZMANNS Theorie des sterischen Ausschlusses der Moleküle der gelösten Substanz von der Oberfläche der Makromoleküle erörtert. Der laut KAUZMANNS Theorie berechnete Wert ist höher als der experimentell gefundene; der Unterschied wird auf Grund des DNA-Modells von WATSON-CRICK erklärt, indem man ihn als Anzeichen dafür ansieht dass, das Makromolekül eine offene Struktur besitzt, welche nicht nur das Eindringen von Wasser, sondern, in geringerem Masse, auch von Saccharose ermöglicht.

Zuletzt wird die Beziehung zwischen diesem Versuchstypus bei multikomponenten Systemen und Messungen der Lichtdispersion und der Gleichgewichtsdialyse erörtert.

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Received March 20th, 1955