A STUDY OF THE INTERACTION BETWEEN CALF THYMUS DESOXYRIBONUCLEIC ACID AND TETRAMETHYLAMMONIUM IONS.

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ABSTRACT

Heat denaturation studies of desoxyribonucleic acid in sodium chloride and tetramethylammonium chloride solutions of low ionic strength have shown that DNA melts at a lower temperature in the TMACl solutions.

Electrophoresis experiments with DNA in a low ionic strength TMACl medium have disclosed that DNA has only a slightly higher mobility in 0.01 F TMACl than in 0.01 F NaCl.

INTRODUCTION

The investigation described in this thesis is one of a series of studies which are concerned with the effects of various cations on the structure and properties of DNA. The long-range hope is that these studies will eventually result in a method of obtaining homogeneous DNA fractions from a heterogeneous DNA.

The tetramethylammonium ion, because of its large size and its single positive charge, has a relatively small charge density; this charge density is less than that of a sodium ion. It was therefore felt that TMA^+ ions would shield the phosphate groups of the DNA less effectively than would Na^+ ions, and less effective shielding of the negative charge of the DNA would result in a destabilization of the DNA with respect to heat. This hypothesis could be checked by comparing the results of heat denaturation experiments in equivalent NaCl and TMACl solutions.

If the DNA did prove to be less stable with respect to heat in the TMACl solution, the neutralization of the charge on the phosphate groups of the DNA molecule would be less in the TMACl. Thus, the effective charge on the DNA molecule would

then be larger in the TMACl. Since the electrophoretic mobility depends in part on the charge of that particle, one would expect the mobility of the DNA to be greater in a TMACl medium than in a NaCl medium. The validity of this line of reasoning could be verified by comparing the mobility of DNA in a TMACl medium with the mobility of DNA in an equivalent NaCl medium.

EXPERIMENTAL METHODS

A. Solutions:

A 0.1 F NaCl solution was prepared from reagent grade Baker and Adamson NaCl. A 0.01 F Tris buffer solution was prepared from Tris-Sigma Company's reagent grade Trizma Base. The pH of the solution was set with reagent grade DuPont HCl. A 0.1 F TMACl was prepared from Matheson Coleman & Bell reagent grade TMACl; the concentration of the TMACl solution was determined by a gravimetric chloride analysis.[1] Final solutions were prepared by appropriate mixing and diluting of these solutions.

Water used in the above solutions and throughout the experiments was redistilled from commercially-distilled water. All glassware used in the experiments was rinsed a number of times with boiling water of this type.

Calf thymus DNA was obtained from the Worthington Biochemical Company. Two separate DNA solutions were prepared, one with NaCl as the salt, the other with TMACl as the salt, by adding small amounts of the solvent to the DNA, DNA-solvent gel, and DNA solution over an extended period of time; both solutions were buffered at pH 7.5 with Tris. Visking tubing was boiled twice in 0.01 F versene, pH 7.6, and twice in the appro-

priate buffered salt solution (0.01 F salt, 0.001 F Tris, pH 7.5), fifteen minutes per change of solution. This tubing was then used in the dialysis of the DNA solutions. The latter were each dialyzed against 0.01 F versene, pH 7.6, for three hours; then against 0.1 F salt solution for 12-16 hours (twice); and finally against the buffered salt solutions for 12-16 hours (three times). The preparation of the DNA solutions was carried on at a temperature of 4°C; the solutions were also stored at this temperature.

The electrophoresis solutions were prepared from reagent grade TMACl, HCl, and Trizma Base obtained from the sources mentioned above; reagent grade Baker and Adamson sucrose was used in preparing the sucrose solutions. Separate salt and buffer solutions were made, desired amounts of which were added to a weighed amount of sucrose and then diluted to make the final solutions. The concentration of the TMACl solution was determined by a Mohr titration analysis.[2] Three drops of chloroform, reagent grade Mallinckrodt, were added to all the sucrose solutions to prevent the growth of organisms.

B. Apparatus:

A Beckman Model 76 pH meter was used throughout the experiments. In measuring the pH's of the DNA solutions examined in the heat denaturation experiments, a Beckman 40316 one-drop electrode and a Beckman 39270 calomel electrode were utilized; this arrangement gives pH measurements reproducible to 0.1 pH unit. A Beckman 41252 glass electrode and a Beckman 39270 calomel electrode were used in determining the pH's of the other solutions used.

The apparatus employed in the heat denaturation experiments was the same used by Dr. W. F. Dove.[3] All spectral measurements were made with the Model 14 Cary spectrophotometer. Solutions to be examined were placed in a Pyrocell microcell, which in turn was placed in a heating compartment made especially for use in the Cary (figure 1). The rate of heating of the compartment was controlled by a Variac. The temperature in the heating cell was given by a Leeds and Northrup Model K-2 potentiometer connected to a copper-constantan thermocouple, one junction of which was situated next to the microcell, the other in water-ice. The thermocouple was calibrated against a thermometer calibrated by the National Bureau of Standards.

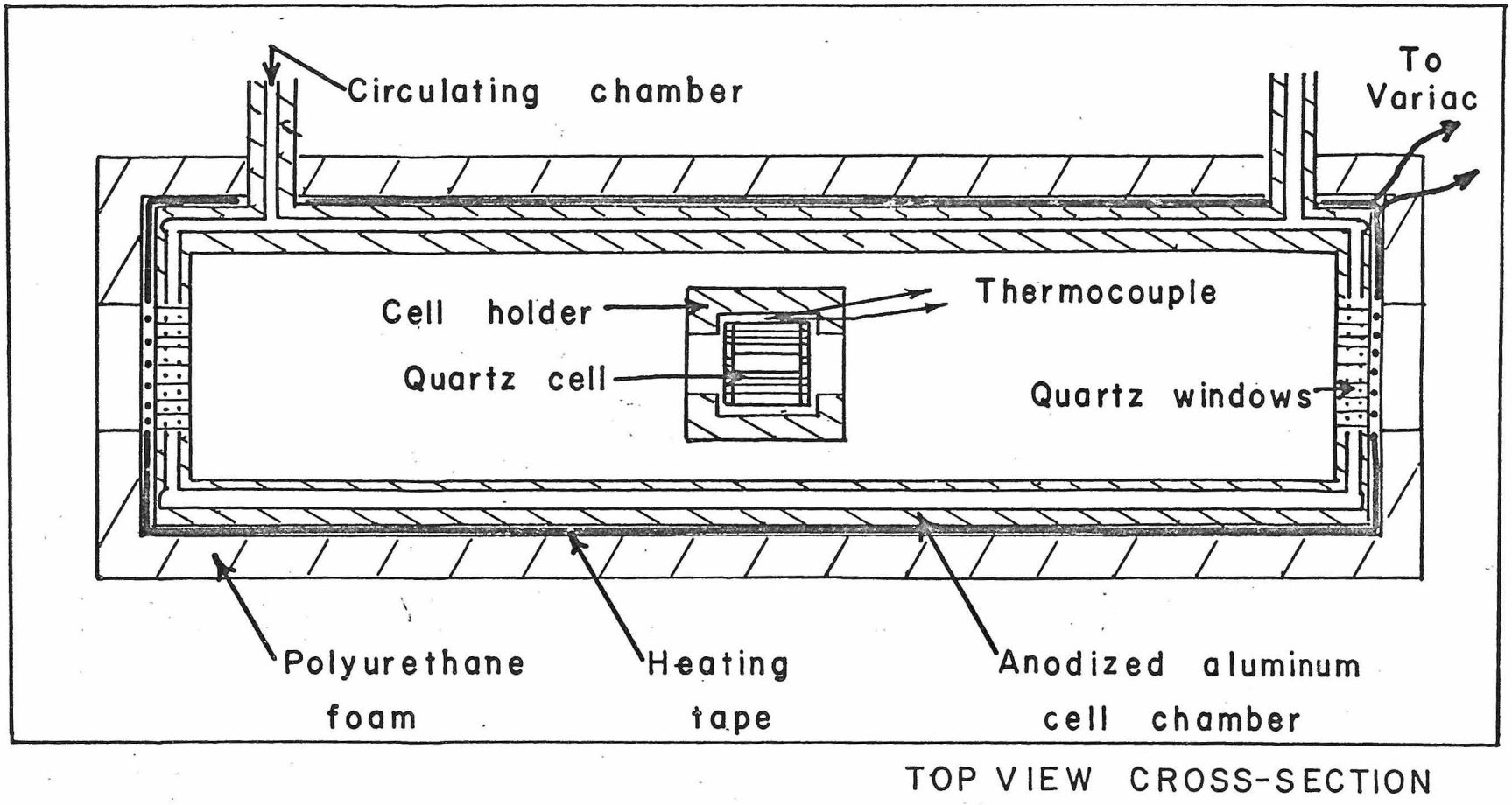

Figure 6. Compartment for heating experiments.

The equipment used in the electrophoresis experiments was the same used by B.M.Olivera et al.[4] The central column of the zone electrophoresis apparatus consists of three concentric quartz tubes: the smallest, for the electrophoresis solutions; the intermediate tube, through which ice water was pumped (this kept the electrophoresis solutions at a temperature of 2°C.); and the outermost tube, a vacuum jacket designed to insulate the central column and prevent the condensation of water on the column. The "arms" of the apparatus (see figure 2) are connected to the innermost quartz tube; the various compartments in the arms contain selected high salt sucrose solutions to prevent any convection between the electrode solutions and the solution in the innermost tube. The solution in the central column is a sucrose density gradient, prepared, quite appropriately, by a density gradient maker.

The platinum electrodes are connected to a 600 volt D.C. power source. The power supply is also connected to an Electro-Methods, Ltd., Low Inertia D.C. Motor and Counter Unit (type 913L) which totals the current passed through the system. The spectral equipment consists of a mercury lamp and a photocell which can be moved

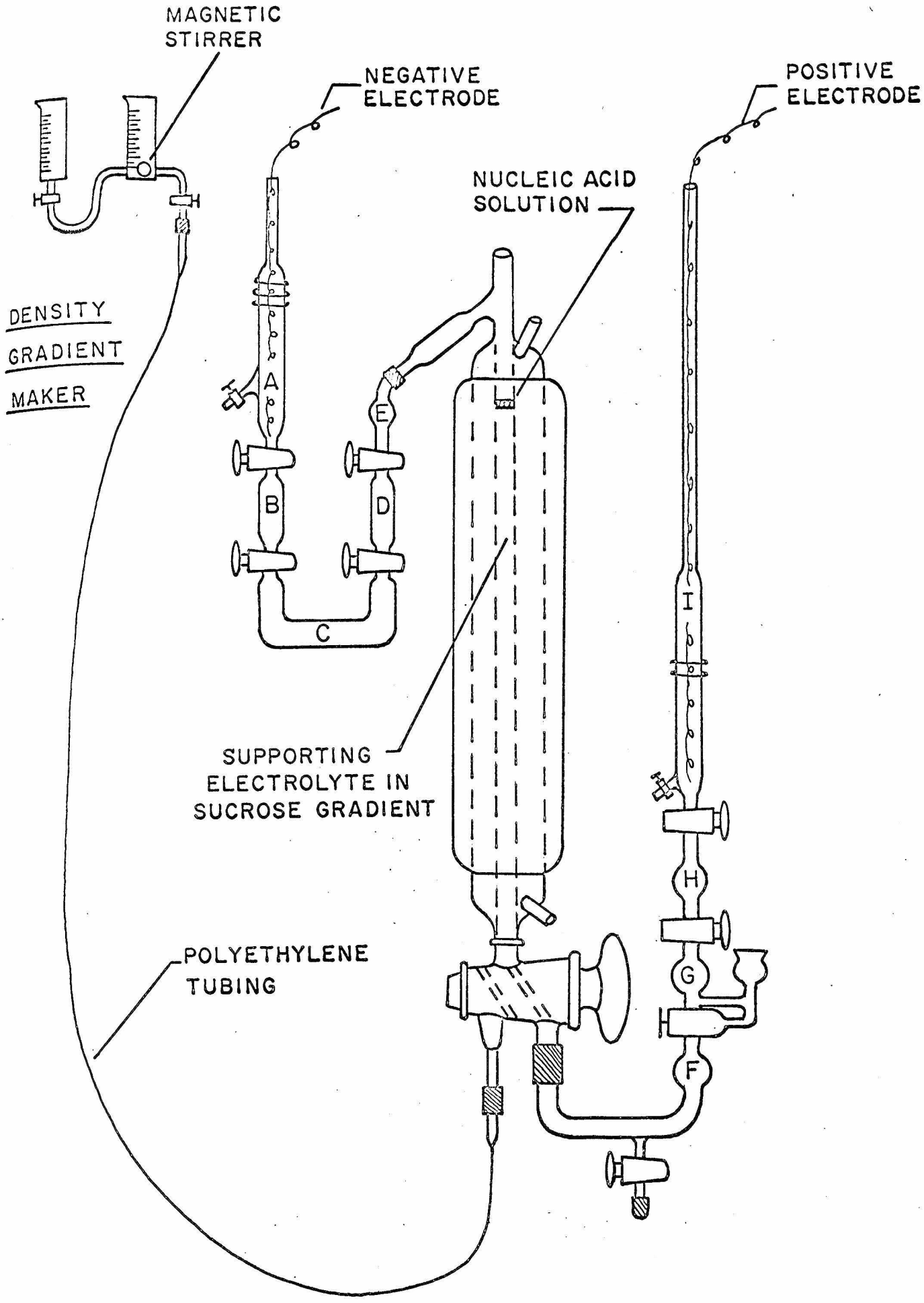

Fig. 1 Olivera, Baine, & Davidson

up and down along the column (figure 3). The photocell is attached to a Keithley Model 151 microvoltmeter and then to a Moseley XY recorder.

The conductivities of the various electrophoresis solutions were measured at 1000 c.p.s. on an Industrial Instruments Conductivity Bridge, Model RC 16B2, using a cell with smooth platinum electrodes. The cell constant was found using a 0.0100 F standard KCl solution.

C. Procedures:

Solutions used in the heat denaturation experiments were prepared by micropipetting 0.100 ml. of a DNA stock solution into 0.90(0) ml. of a buffered salt solution (or water) in a 1 ml. volumetric flask. About 0.7 ml. of the resulting solution was transferred to the microcell, placed in a near-vacuum to remove dissolved air, and finally put in the heating compartment, after the microcell was capped with a glass stopper. The remaining solution was used to measure the pH of the solution in the one-drop electrode.

After an initial spectrum, the Variac was set to provide a constant heating rate of 0.5 deg./min. Spectra were taken at suitable intervals; potentiometer readings were recorded at 260 mμ. When further heating produced no change in the

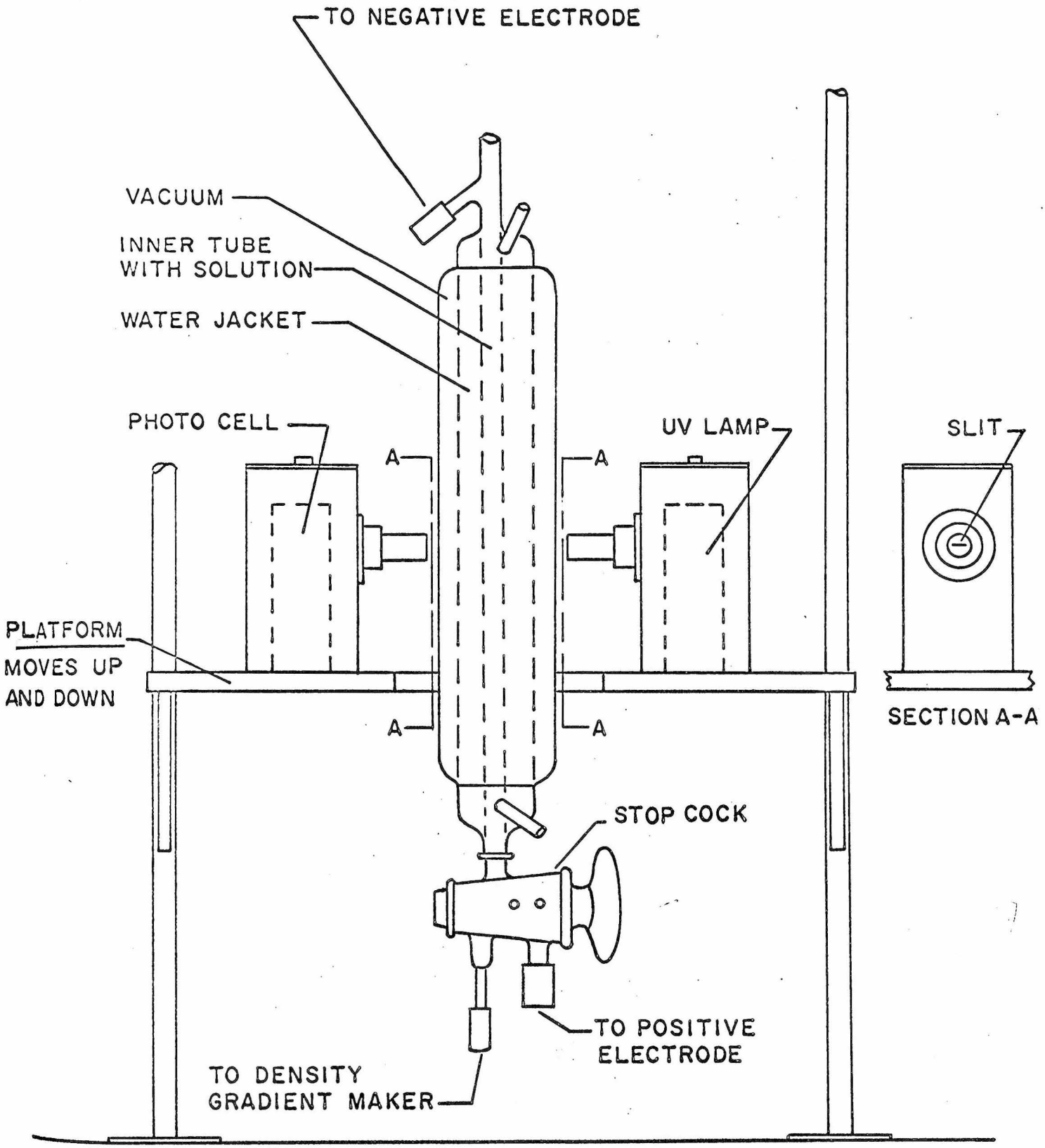

Fig. 3

Olivera Bayne and Dain

optical density of the solutions, they were allowed to cool and their pH's were rechecked. Blanks were run in the heating compartment, and appropriate adjustments were made in the spectra. The optical density at 260 mμ was measured from the Cary spectra. Hyperchromicity$_{260}$ vs. temperature graphs were prepared.

The arms of the electrophoresis apparatus were filled as follows (see figure 2): compartments A and I, 0.1 F TMACl, 0.2 F Tris; B,D, and H, 0.2 F TMACl, 0.02 F Tris, 5% (by weight) sucrose; C and G, 0.2 F TMACl, 0.02 F Tris, 15% sucrose; F, 0.01 F TMACl, 0.001 F Tris, 25% sucrose. The central column was filled to near the top with a sucrose density gradient prepared by the density gradient maker; this solution was 0.01 F TMACl, 0.001 F Tris, pH 7.4, 5%-20% sucrose. The solution in the central column was allowed to stabilize for 1½-2½ hours.

By mixing the appropriate amounts of different stock solutions, a DNA solution of 3% sucrose, 0.01 F TMACl. 0.001 F Tris, pH 7.4, and approximately O.D. one was prepared. About 0.2 ml. of this solution was placed on top of the sucrose gradient, and then the rest of the tube was carefully filled with 0% sucrose, 0.01 F TMACl, 0.001

F Tris, pH 7.4, solution. An initial spectrum was taken, after which the power was turned on. The column was scanned at regular intervals, and the reading on the coulomb counter was noted when the spectral equipment recorded the peak corresponding to the DNA band.

In the electrophoresis run using denatured DNA, the DNA was denatured by leaving the 3% sucrose DNA solution which had been prepared in a beaker containing water at 80-90°C. for fifteen minutes. The DNA solution was then immersed in ice water for five minutes before being transferred to the electrophoresis tube. The rest of the procedure was the same as for native DNA. The solutions in the arms were not changed between runs, but the stopcocks were closed to prevent mixing. d (distance the DNA peak travelled) vs. Q (coulombs passed through the system) graphs were prepared.

Conductivity measurements of the solutions used in the central column were made on the conductivity bridge at 0°C. These measurements were highly sensitive, and were reproducible only if a very standardized measurement procedure was painstakingly followed.

RESULTS

A. Heat denaturation experiments:

Graphs were prepared showing the hyperchromicity at 260 mμ vs. the temperature at 260 mμ. The hyperchromicity equals the ratio of the optical density (O.D.) at time t to the initial O.D.; the potentiometer readings were converted to temperatures with a calibration chart prepared using a National Bureau of Standards thermometer. The temperature at half the total hyperchromicity is designated as T_m, the mid-point melting temperature of the DNA.

The following data refers to calf thymus DNA subjected to heat denaturation in the various solutions listed:

Solution	pH	T_m (°C.)
0.0100 F NaCl, 0.00100 F Tris	7.4	68.0
	7.2	68.0
0.0108 F TMACl, 0.00100 F Tris	7.4	64.4
	7.3	63.2
0.00100 F NaCl, 0.00010 F Tris	6.8	51.5
	6.9	50.5
0.00108 F TMACl, 0.00010 F Tris	6.8	46.0
	6.9	46.9
0.00972 F TMACl, 0.00100 F NaCl, 0.00100 F Tris	7.2	66.7
	7.2	65.9

The T_m's of the NaCl solutions agree quite closely with those obtained by Dove.[3]

The T_m varies linearly with the log of

the ionic strength of the solution; thus, a 0.0108 F NaCl, 0.00100 F Tris solution, pH 7.3, would have a T_m of 69°C., and a 0.00108 F NaCl, 0.00010 F Tris solution, pH 6.9, would have a melting point of 52°C. Consequently, there is a 4-5°C. difference in the melting temperature between calf thymus DNA in a NaCl solution and calf thymus DNA in the corresponding TMACl solution.

The difference between the T_m's of 0.01 F NaCl and 0.001 F NaCl is essentially the same as the difference between the T_m's of 0.01 F TMACl and 0.001 F TMACl.

The denaturation of DNA in the mixed solvent shows quite clearly that the change in T_m is not linear with the change in the Na^+ concentration.

B. Electrophoresis experiments:

Graphs were prepared showing d vs. Q. d is the distance the DNA peak travelled down the electrophoresis column from the initial position to the position at time t; markers are spaced along the column at specific distances and are recorded along with the DNA peak, thereby allowing the distance down the tube to be measured. Q is the coulombs of electricity passed through the

column from the initial time to the time t, as computed from the Electro-Methods counter. These graphs give a nearly linear curve; the slope of the curve, d/Q, is used in calculating the mobility of the DNA down the column, U. The mobility is defined as U = v/E, where v is the velocity of the DNA proceeding down the column and E is the voltage per cm. through the column. Where t is the time, I is the current through the column, R is the resistance per cm. in the column, and Q is the number of coulombs passed through the column,[4]

$$U = \frac{v}{E} = \frac{v}{IR} = \frac{vt}{IRt} = \frac{d}{QR} .$$

The resistances of the electrophoresis solutions were obtained through conductivity measurements of the solutions. Since the temperature presumably has the same fractional effect on the mobilities of all ions, the conductivities were measured at 0°C., and the R of the electrophoresis solution at 0°C. was used in calculating U. Also, U is ideally independent of the sucrose concentration, so the R of the 0% sucrose electrophoresis solution was used in calculating U. Thus, the following U's are calculated at 0°C. and 0% sucrose:

Solution	Electrophoretic Mobility (U)
Native calf thymus DNA, 0.01 F NaCl, 0.001 F Tris, pH 7.5	2.17×10^{-4} cm^2/volt-sec
Native calf thymus DNA, 0.01 F TMACl, 0.001 F Tris, pH 7.4	2.19×10^{-4}
Denatured calf thymus DNA, 0.01 F NaCl, 0.001 F Tris, pH 7.5	1.90×10^{-4}
Denatured calf thymus DNA, 0.01 F TMACl, 0.001 F Tris, pH 7.4	1.92×10^{-4}

The electrophoresis runs in NaCl were done by B.M.Olivera.[4] Reproducible conductivity measuremets were obtained for only two runs in TMACl, and they are the ones noted just above. Two other runs, one with native calf thymus DNA and one with denatured, gave d/Q values which were within 3% of the respective d/Q values for the runs noted above, but a reproducible R value could not be found. Electrophoresis runs in TMACl to check the reproducibility of the above mobilties are desirable.

DISCUSSION

The heat denaturation experiments reveal that the DNA melts at a lower temperature in the TMACl solutions. This destabilization with respect to heat can be explained in four ways:

1.) The TMA^+ ion, being much larger than the Na^+ ion, is not accomodated as easily into the DNA structure. Thus, the Na^+ ions stabilize the DNA more because they are able to neutralize charges on the anionic DNA molecule in places that are inaccessible to the larger TMA^+ ions. This might be called a steric effect.

2.) Because of their smaller size, more Na^+ ions can position themselves along a given length of the DNA chain than can Na^+ ions. Consequently, more of the phosphate groups are neutralized, and the DNA molecule is more stable.

3.) The electrostatic binding is more effective with Na^+ ions because of a Coulomb's Law effect. Because of their size, the TMA^+ ions cannot approach the DNA molecule as closely as the Na^+ ions; this results in less binding, since the distance between the negative and positive centers is

greater. This is the conventional explanation for why Na^+ ions are generally more tightly bound by complexing anions than are TMA^+ ions.

4.) The DNA can distinguish between Na^+ and TMA^+ ions and selectively binds Na^+ ions to the DNA bases, rather than the phosphates. This binding makes the DNA more stable in a NaCl solution.

The relative importance of the above theories can be determined to a small degree by considering the following. The fourth explanation can be virtually discounted because previous work has shown that Na^+-DNA binding is an electrostatic binding between the Na^+ ions and the phosphate groups of the DNA molecule.[3] The heat denaturation experiments in the mixed solvent (0.00972 F TMACl, 0.00100 F NaCl, 0.00100 F Tris) show that the second explanation is not the cause of the destabilization effect, since one would then expect a linear change in T_m as the salt solution changes from TMACl to a mixed (TMACl-NaCl) solvent to NaCl. Also, the distance between the phosphate groups (6.6 A.) makes this explanation implausible. The importance of the first reason given above is questionable since it is not clear just how many places the Na^+ ion will go

that the TMA^+ ion will not go. Thus, the destabilization can be explained primarily by the third reason given above, though the other three, especially the first, may be included in accounting for the entire effect.

In short, the DNA molecule is less stable in a TMACl solution than in a NaCl solution because the TMA^+ ions are less effective in shielding the negative charges of the phosphate groups of the DNA molecule. This loss of effectiveness in TMACl is due to a combination of electrostatic and position factors.

Given the above destabilization effect, the electrophoresis results are in the right direction: the mobility of the DNA is slightly greater in the TMACl medium than in the NaCl medium. This follows from the larger effective charge of the DNA molecule in a TMACl solution. However, the difference between the mobilities of the DNA in the two solutions (NaCl and TMACl) is barely significant, and certainly not as much as expected. Yet the insignificance of the difference between the mobilities may be attributable to other factors.

Electrophoresis experiments performed by B.M.Olivera on calf thymus DNA in NaCl at varying pH's reveal that the electrophoretic mobility of

the DNA does not change as much as one would expect it to after considering the titration curve of the DNA.[4] One hypothesis advanced to explain this was the following: Na^+ ions are tightly bound to the DNA and are dragged along as the DNA moves towards the positive electrode. Titration to a low pH mostly removes one of these tightly-bound Na^+ ions by replacement with a $-NH_3^+$ group. Consequently, when most of the hydrogen ions added to the DNA merely "replace" Na^+ ions, the effective charge of the DNA molecule, and thus its mobility, does not change much when the pH is changed.

But the electrophoresis experiments of calf thymus DNA in TMACl indicate that the above hypothesis is incorrect, at least for low concentrations of TMACl. Since the difference in the mobilities in the two media is not large enough to be very significant, one can assume that TMA^+-DNA binding is qualitatively the same as Na^+-DNA binding. Previous work has shown that TMA^+ ions are weakly bound to phosphate groups.[5] Thus, the interaction between DNA and Na^+ or TMA^+ ions can be described as a relatively weak, non-selective, electrostatic attraction between the cations and the negatively charged DNA molecule. This contradicts the hypothesis

stated above, which was based on the premise that Na^+ ions are strongly bound to the phosphate groups of the DNA.

P.D.Ross and his associates have performed electrophoresis experiments on DNA in both NaCl and TMACl media.[6,7] Their experiments show a much greater difference between U in NaCl and U in TMACl than do the experiments described in this report. This discrepancy is explained by two considerations.

First, the Ross group used a free boundary (Tiselius) electrophoresis setup rather than a zone electrophoresis apparatus. This has resulted in mobilities which are approximately 20% higher than the corresponding mobilities obtained from the zone electrophoresis apparatus. Also, the data obtained on the Tiselius setup have a relatively large amount of uncertainty, especially at low concentrations.

Second, the experiments of the Ross group were carried out at higher concentrations of salt. And furthermore, the mobilities of Ross et al. do not vary linearly with the log of the ionic strength as do the mobilities obtained on the zone electrophoresis apparatus by B.M.Olivera.[4] Thus, the results of Ross et al. and the results of Davidson et al. do not correlate, but they are not really comparable.

Ross hypothesizes that the difference between his U in NaCl and his U in TMACl values is due to site binding of the Na^+ ions to the DNA, a phenomenon not exhibited by the TMA^+ ions. One would expect this specific binding to decrease as the concentration of Na^+ ions is lowered, and Ross' results do show a slight decrease in the U_{TMA^+}/ U_{Na^+} ratio with the lowering of the ionic strength. It is quite conceivable that the U_{TMA^+} / U_{Na^+} ratio could be nearly one at cation concentrations of 0.01 F on the Tiselius apparatus as they are on the zone electrophoresis apparatus. However, the electrophoresis results in TMACl obtained on the zone electrophoresis apparatus have to be verified and runs at different concentrations of TMACl have to be made, and the uncertainty and non-linearity of Ross' results must be accounted for, before any comparison and correlation of the mobilities obtained on the two different types of apparatus can be made.

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