STUDIES OF THE METAL ION COMPLEXES OF PURINES AND PYRIMIDINES BY ELECTROPHORESIS AND OTHER METHODS

Thesis by
Janis Vasilavskis

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I am grateful to Dr. N. Davidson for his guidance and help. It has been a rewarding experience to work with him and the students in his group.

Mr. J. Kasper made very substantial contributions to the design and construction of the electrophoresis apparatus.

I am indebted to the National Science Foundation, the Sloan Foundation, the Atomic Energy Commission, and to Caltech for financial support.
ABSTRACT

A density-gradient-stabilized electrophoresis apparatus has been designed and built.

Spectrophotometric and electrophoretic measurements are used to investigate the hydrogen ion, mercuric ion, and methylmercuric ion complexes of purine and pyrimidine derivatives. The results are used to infer the charge of the complexes.

The problem of determining the charge of an ion from its mobility and diffusion coefficient is also considered.
I. INTRODUCTION

Ferreira et al. (1) (hereinafter referred to as PBZYVD) used spectrophotometric measurements and pH-stat titrations to investigate the complexes formed by purine and pyrimidine derivatives in the presence of excess mercuric ion. The measurements were made in the pH range of 1-3 with Hg\(^{+2}\) concentrations of 10\(^{-2}\) to 10\(^{-3}\) M and ligand concentrations in the 10\(^{-4}\) M range. Under the conditions one might expect the following general reaction to take place

\[
HP^{+n} + Hg^{+2} \rightleftharpoons P-Hg^{+(n+1)} + H^+ \tag{1}
\]

where HP refers to a purine or pyrimidine derivative having a charge \(+n\).*

However the experiments seemed consistent with the postulate that hydrolysis of the complexed mercuric ion was taking place,

\[
HP^{+n} + Hg-OH^{+2} \rightleftharpoons P-Hg-OH^{+n} + 2H^+ \tag{2}
\]

The adenine, 7-methyladenine, 9-methyladenine and 6-N,N-dimethylaminopurine systems seemed to give the hydrolyzed product according to reaction (2) while in the cytidine case both reactions (1) and (2) appeared to be taking place giving a mixture of products.

From the above results one infers that the acid pK for the reaction

\[
P-Hg-OH^{+n} \rightleftharpoons P-Hg-OH^{+n} + H^+ \tag{3}
\]

is less than 1.5. This result is surprising, in view of the fact that the pK\(_a\) of H\(_2\)O-Hg-OH\(^{+2}\) is 3.7 (2) and the pK\(_a\) of H\(_2\)O-Hg-OH\(^+\) is 2.6 (2) while

---

*The molecular structures of the purines and pyrimidines to be discussed in this thesis are shown in Figure 1. One should also note the numbering systems and the acid pK's.
Fig 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>pK's</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>H</td>
<td>H</td>
<td>4.22</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Sugar</td>
<td>H</td>
<td>3.45</td>
</tr>
<tr>
<td>9-Methyladenine</td>
<td>CH₃</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine</td>
<td>H</td>
<td>CH₃</td>
<td></td>
</tr>
<tr>
<td>7-Methyladenine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytidine</td>
<td></td>
<td></td>
<td>4.22</td>
</tr>
<tr>
<td>Deoxyadenylic acid (5')</td>
<td></td>
<td></td>
<td>3.3</td>
</tr>
</tbody>
</table>

- N: Nitrogen, CH₃: Methyl group
the pKₐ of CH₂-Hg-O₂⁻ is 4.5 (3,4). Thus it appears that the acidity of the R-Hg-O₂⁻ group is enhanced when R is either a purine or a pyrimidine derivative.

There are several reasons why the FBZYVD results may not be completely reliable:

(a) The solutions used in the pH stat experiments already have a relatively high acid concentration so a significant error may exist in the determination of the amount of hydrogen ion liberated when the complexes are formed.

(b) The conclusions drawn from the spectrophotometric experiments depend on plotting some function of the change in absorbance and finding linear behavior with respect to some function of H⁺ and Hg²⁺. From the plot one must obtain both the extinction coefficient of the complex and the constant K_c for complex formation. The procedure becomes unreliable if not impossible if two or more reactions are taking place simultaneously. Thus for the reactions

\[ \text{HP}^{n+} + \text{Hg}^{+2} \leftrightarrow \text{P-Hg}^{+(n+1)} + \text{H}^+ \quad \Delta A = f \left( \frac{\text{H}^+}{\text{Hg}^{+2}} \right) \]

and

\[ \text{HP}^{n+} + \text{HgOH}^{+2} \leftrightarrow \text{P-Hg-OH}^{+n} + 2\text{H}^+ \quad \Delta A = g \left( \frac{(\text{H}^+)^2}{\text{Hg}^{+2}} \right) \]

the change in the absorbance A is a more complicated function of H⁺ and Hg²⁺ which does not lend itself to simple plotting and interpretation. The analysis must give the extinction coefficients for both complexes and the equilibrium constants for both reactions.

As a further complication there is also the possibility of the following type of reaction

\[ \text{HP}^{n+} + 2\text{Hg}^{+2} \leftrightarrow \text{P-(Hg)}^{+(n+4-m)} + m\text{H}^+ \]
In view of the above discussion it was felt that some other type of confirmation for reaction (2) would be desirable.

One piece of information about a complex which would be helpful in elucidating its formula is its charge, or in the case of a mixture, the average charge. This thesis principally describes an electrophoretic study of these complexes aimed at the determination of their charge.

There are two possible methods of accomplishing the above

a) Theoretical calculation of the charge of a complex from measurements of its mobility and diffusion constant, and

b) Determination of the charge by comparison of the electrophoretic mobilities of structurally similar ions.

Method (a) makes use of the following relationships

\[ u = \frac{Ze}{f} \quad (5) \]

\[ Z = \text{ionic charge} \]

\[ e = \text{electronic charge} \]

\[ f = \text{frictional coefficient} \]

and

\[ D = \frac{kT}{f} \quad (6) \]

\[ D = \text{diffusion constant} \]

\[ k = \text{molecular gas constant} \]

\[ T = \text{absolute temperature} \]

Therefore

\[ \frac{u}{D} = \frac{Ze}{kT} \quad (7) \]

or

\[ Z = \frac{u kT}{De} \quad (8) \]

so that a measurement of \( u \) and \( D \) makes it possible to calculate \( Z \).

As discussed later, the above relationships do not hold accurately at a finite salt concentration. Nevertheless we endeavored to build an electrophoresis apparatus that would measure \( u \) and \( D \) in order to test equation (8) under practical circumstances.
However at its present stage of development the apparatus is satisfactory for measuring \( u \) to one or two per cent, but it is not good enough to determine \( D \) to better than 30%. We have therefore not been able to make a decisive study of the use of equation (8) for charge determination, although our semiquantitative results appear promising.

Method (b) makes use of the relation

\[
u = \frac{Ze}{\pi}
\]  

(9)

One might guess that, for example, for the ions

\[
\begin{align*}
&
\begin{array}{c}
\text{NH}_2 \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{R}
\end{array} \\
&
\text{(I)}
\end{align*}
\]

\[
\begin{align*}
&
\begin{array}{c}
\text{NH}_2 \\
\text{Hg} \\
\text{N} \\
\text{N} \\
\text{R}
\end{array} \\
&
\text{(II)}
\end{align*}
\]

\[
\begin{align*}
&
\begin{array}{c}
\text{NH}_2 \\
\text{Hg-CH} \\
\text{N} \\
\text{N} \\
\text{R}
\end{array} \\
&
\text{(III)}
\end{align*}
\]

the geometries are similar and therefore the friction coefficients would be similar. It should be noted that we know that protonated adenine or adenosine (I) has a charge of +1. It was therefore hoped that by comparison of the mobility of the mercury complex with that of the protonated form at different pH's it would be possible to infer the charge of the mercury complexes.

The measurements reported in this thesis confirm some of the results obtained by FBZYVD. However in several cases (notably the Hg\(^{+2}\)-adenine
system) the electrophoresis data strongly suggest that in the pH range where FEZYVD worked significant amounts of both the P-Hg-OH\(^{+n}\) and P-Hg-OH\(_2\)^{+(n+1)} complexes were present.

The methyl mercury cation, CH\(_3\)-Hg\(^+\), is in some respects easier to work with than is Hg\(^{2+}\). It is monofunctional and it does not precipitate at high pH. As a supplement to the electrophoretic study of the CH\(_3\)-Hg\(^+\)-adenine complexes, a careful spectrophotometric investigation of this system was performed. It was discovered that the following ions exist

\[
\begin{array}{cccc}
\text{NH}_2 & & & \\
\text{CH}_3\text{-Hg} & & & \\
\text{N} & & & \\
\text{H} & & & \\
\end{array}
\]  
\begin{array}{ccc}
\text{NH}_2 & \text{Hg-CH}_3 & + \\
\text{N} & & \\
\text{Hg-CH}_3 & & \\
\end{array}
\begin{array}{ccc}
\text{NH}_2 & \text{Hg-CH}_3 & 0 \\
\text{N} & & \\
\text{Hg-CH}_3 & & \\
\end{array}
\]  

(IV) (V) (VI)

and their electrophoretic mobilities have been measured. The above series of complexes provides a very helpful confirmation of the semiquantitative correlation between electrical mobility and charge.
II. APPARATUS

a. General

A density-gradient-stabilized electrophoresis apparatus has been designed and built. The concentration profile of the material of interest is observed by the use of an ultraviolet optical scanning device. The density gradient is necessary because the concentrations of the absorbing complexes are so low that convection due to heating would disturb the electrophoretic boundaries even at low electric fields. The ultraviolet scanning device is a convenient detection system because all of the purine and pyrimidine derivatives and their complexes have an absorption maximum in the region of 250 to 280 μm.

Figure 2 is a schematic drawing of the apparatus. The light from the mercury arc (a) passing through a quartz diffuser (b) falls upon a diaphragm type slit (c). The iris of the diaphragm appears as the effective light source for the rest of the optical system. The light from the iris passes through a series of filters (d), (e) and (f) making it essentially monochromatic (265 μm), and the quartz lens (g) makes it parallel. The light then enters the cooling cell (i) and passes through the optical section of the electrophoresis cell (j). The lens (s) forms an image of the electrophoresis cell at (m) where a horizontal scanning slit (n) can be moved vertically. As the slit is moved light from different horizontal sections of the cell falls on lens (n) which focuses it on the same photosensitive part of the phototube no matter where the scanning slit is positioned. Any change in the position of the slit is translated, by means of a potentiometer, into a corresponding change in current which is then plotted against
Fig 2

Electrophoresis Apparatus
(Schematic)

to battery
and x-y recorder

to power
supply and
x-y recorder

---

a - mercury arc
b - quartz light diffuser
c - slit
d,e,f - filters
g,l,n - quartz lenses
h,k - quartz windows
i - cooling cell
j - electrophoresis cell
k - moving slit
l - phototube

---

1.8 cm | 9 cm
4 cm | 5 cm
8 cm | 8 cm
30.3 cm

---

defogging
insulation
defogging

---

14.8 cm
52.7 cm
60 cm

---

30 cm

---

3.8 cm

---

15.5 cm

---

8 cm
the photocell current by use of an x-y recorder. Thus one obtains a plot of the intensity of light passing through the electrophoresis cell versus the position in the cell. A more detailed description of the apparatus appears in Appendix I.

The space resolution attainable at the present is 0.2 mm. Absorbances in the range of 0.1 to 2 in the 240-280 nm region can be measured fairly accurately.

b. Electrophoresis Cell

A schematic drawing of the cell is shown in Figure 3. A is one of the electrode compartments. Extending 4 cm into A is a 12.0 cm long 1.0 cm square glass tube. From points B to C it is made of quartz. The rest of the tube as well as the rest of the cell is opaque to ultraviolet light. The quartz was secured to the glass by gluing several layers of 1 cm wide A strips around the butt joints. Epoxy resin had to be used (Resiweld no. 4). Thus the optically acceptable part of the cell is 4 cm long.

Hooked on in front of the cell is a 4 mm wide vertical slit D with 0.125 mm diameter wire position markers spaced exactly 5 mm apart. These markers then give discontinuities in the intensity plot thus giving precise position (see Fig. 4, page 19).

Just below joint C a short glass capillary E is glued into the wall of the cell. It has an i.d. of about 1.5 mm and extends about 3.5 mm into the cell. The size of the capillary and its position are fairly critical because the boundary and density gradient are passed into the cell here. Skew boundaries are obtained if one is not careful with the above specifications.
Fig 3

Electrophoresis Cell

- Ag-AgCl electrode
- 0% D₂O solution (no. 7)
- 4% D₂O solution (no. 6)
- 8-18% D₂O gradient (no. 2, 4)
- wire markers
- 18-28% D₂O gradient with absorbing material (no. 3, 5)
- inlet tube
- density gradient machine
- electrolyte passed in here
- 8-28% D₂O gradient passed through here (no. 2-5)
- stopcocks
- electrolyte (no. 8)
- Ag-AgCl electrode
Attached to E is a length of P.E. 100 intramedic polyethylene tubing which leads to the mixer and then to the density gradient and boundary making machine.

From point F on there is a 100 cm long extension of the cell G made from $\frac{1}{2}$" o.d. glass tubing. This extra length is necessary when doing electrophoresis in acid or alkaline solution to guard against the gain or loss of the fast moving $H^+$ and $OH^-$ ions. For convenience in filling the cell and removing air from it there are glass stopcocks at positions H, I, and J. K is the second electrode compartment.

The electrodes are not permanently sealed in. They are Ag-AgCl and can be regenerated and replaced as necessary. The electrodes are connected to a Lambda Electronics Corp. 300 volt DC power supply and the current passing through the cell is measured with a variable range milliammeter (Sensitive Instrument Corp.).

A thermocouple probe was made for measuring the temperature inside the electrophoresis cell and a similar Ag-AgCl electrode probe for actually measuring the voltage drop. These probes were used only in certain cases for their presence tends to disturb the boundary somewhat.

c. Density Gradient Machine and Mixer

The density gradient and boundary making machine consists of two cams of radii $r_1 = 2 + 1.26 \frac{\theta^2}{(270)^2}$ cm and $r_2 = 2 + 2.52 \frac{\theta}{270} - 1.26 \frac{\theta^2}{(270)^2}$ cm where $\theta$ varies between 0 and 270 degrees. As the cams are driven by a motor they push in the plungers of two syringes containing solutions of density $\chi$ and $\gamma$ respectively so that a linear density gradient is obtained upon mixing. The mixing of solutions $\chi$ and $\gamma$ has to be done in as small a volume as possible. This is accomplished by having the syringes connected by .034" i.d.
polyethylene tubing to a T-tube of similar dimensions. Then the solution passes through a helix of 15 turns and 1.2 cm diameter of the above type tubing and then into the cell. Most of the mixing takes place in the helix. The speed of the motor driving the cams is just as critical as the position of the inlet tube in the electrophoresis cell since it determines the speed with which the solution enters the cell.

To form an electrophoresis boundary one has two sets of syringes containing solutions of the same densities and compositions \( x \) and \( y \) but only one set containing the absorbing material. At the half-way point in the gradient one simply exchanges the syringes. Thus one obtains a boundary with negligible density discontinuity. The linearity of the density gradient and the completeness of mixing were confirmed by experiment.
III. EXPERIMENTAL PROCEDURE

a. Materials and Stock Solutions

Table 1 lists all of the materials used in making the necessary stock and experimental solutions. The experimental solutions were usually made by mixing appropriate amounts of stock solutions and then diluting to the desired concentration. The solutions in general were made just before the experiments. Doubly distilled water was used in all cases.

Stock solutions of all the purine and pyrimidine derivatives, Hg$^{++}$, NaAc, NaCl, NaClO$_4$, Tris buffer and sucrose were made by weighing out the respective chemicals.

The mercuric oxide was dissolved in excess HClO$_4$ of known concentration. The presence of excess acid is required to prevent hydrolysis of Hg$^{++}$.

The acids were standardized with the standard base obtained from the Bio-Rad Labs.

The concentration of CH$_3$-Hg-OH was determined by HClO$_4$ titration in presence of Cl$^-$. The results were reproducible within one part per thousand.

The DNA concentration was determined from the absorbance at 260 nm, assuming $\varepsilon_{260} = 6.5 \times 10^3$.

All of the purine and pyrimidine stock solutions and also any solutions containing sucrose were kept refrigerated at all times in order to minimize bacterial growth.

It was found that some of the complexes decompose in the presence of ultraviolet light. Therefore all solutions containing purines or
<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Purity and Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>Puritas</td>
<td>Doubly distilled</td>
</tr>
<tr>
<td>D₂O</td>
<td>Liquid Carbonic-Division of General Dynamics Corp.</td>
<td>Batch XIX; &gt; 99.5%</td>
</tr>
<tr>
<td>HCl</td>
<td>Braun Corp.</td>
<td>Conc.; R.G.</td>
</tr>
<tr>
<td>HClO₄</td>
<td>Mallinckrodt</td>
<td>60%; A.R.</td>
</tr>
<tr>
<td>NaOH</td>
<td>Baker &amp; Adamson</td>
<td>Pellets; R.G.</td>
</tr>
<tr>
<td>NaOH</td>
<td>Bio-Rad Labs.</td>
<td>Stand. 0.1N; CO₂ free</td>
</tr>
<tr>
<td>CH₃COOH</td>
<td>Baker</td>
<td>Glacial, A.R.</td>
</tr>
<tr>
<td>CH₃-Hg-OH</td>
<td>Agricultural Div. Morton Chem. Co.</td>
<td>21.5% Hg; 0.2% NaNO₂</td>
</tr>
<tr>
<td>HgO</td>
<td>Baker &amp; Adamson</td>
<td>R.G.</td>
</tr>
<tr>
<td>CH₃COONa</td>
<td>Mallinckrodt</td>
<td>Anhydrous; A.R.</td>
</tr>
<tr>
<td>NaCl</td>
<td>Baker &amp; Adamson</td>
<td>R.G.</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>C. F. Smith Chem. Co.</td>
<td>Anhydrous</td>
</tr>
<tr>
<td>Tris(hydroxymethyl)- aminomethane</td>
<td>Sigma Chem. Co.</td>
<td>R.G.; 111B-122-3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Baker &amp; Adamson</td>
<td>R.G.</td>
</tr>
<tr>
<td>Adenine</td>
<td>Cal. Biochem.</td>
<td>Lot 102915</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Cal. Biochem.</td>
<td>Lot 510431</td>
</tr>
<tr>
<td>Deoxyadenylic acid(5') di Na salt</td>
<td>Cal. Biochem.</td>
<td>Lot 730232</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine</td>
<td>Cal. Biochem.</td>
<td>Lot 520502</td>
</tr>
<tr>
<td>7-Methyladenine</td>
<td>Cyclo Chem. Corp.</td>
<td>Lot K1163</td>
</tr>
<tr>
<td>9-Methyladenine</td>
<td>Cyclo Chem. Corp.</td>
<td>Lot A1075</td>
</tr>
<tr>
<td>Cytidine</td>
<td>Cal. Biochem.</td>
<td>Lot 430345</td>
</tr>
<tr>
<td>DNA-calf thymus</td>
<td>Worthington Biochem. Corp.</td>
<td>&quot;highly polymerized&quot;; native</td>
</tr>
</tbody>
</table>
pyrimidines complexed either with Hg$^{++}$ or CH$_3$-Hg$^+$ were kept in the absence of light.

For the initial experiments sucrose was used to provide a density gradient. However this practice was discontinued because a) it was found that sucrose absorbs enough in the 240-280 μm range to make the determination of the initial intensity troublesome and b) it was observed to react with Hg$^{+2}$ ion thus making it useless for density gradient formation for most of the complex systems that were investigated. A further complication was the large schlieren effect obtained upon sharpening of the initial boundary by careful removal of the solution from the initial boundary which sometimes had a spread between 1 and 5 mm. In general sharpening is necessary only for the diffusion measurements.

Substitution of D$_2$O for sucrose eliminated the first two problems completely and reduced the last one considerably since the refractive indices of H$_2$O and D$_2$O are very similar.

b. Electrophoresis Solutions

The important general point is that the velocity of a moving boundary is measured for a boundary between a solution containing for example 0.1 F NaClO$_4$, 0.01 F HClO$_4$, 0.001 F Hg$^{++}$, and a second solution containing the same concentrations of these constituents and 0.0001 F purine. Thus, as the purine constituent migrates it encounters an essentially constant concentration of Hg$^{++}$ and H$^+$, so that the equilibria involved in the formation of the complex ions are not disturbed. Furthermore, because of the relatively low concentration of the charged purine constituent the concentration changes for the supporting electrolyte and the conductance change at the boundary will be negligible.
Table 2 lists the compositions of a representative set of solutions used in an electrophoresis experiment. For the following discussion refer also to Figure 3 which shows the position of the different solutions for a typical experiment. The procedure for filling the electrophoresis cell is described in some detail in Appendix 2. It is identical for all experiments and should be rigidly adhered to to guarantee the best results.

**TABLE 2**

A Representative Set of Solutions for an Electrophoresis Experiment

<table>
<thead>
<tr>
<th>Soln. no.</th>
<th>$D_2O$ vol. %</th>
<th>$NaClO_4$ $F$</th>
<th>$HClO_4$ $F \times 10^3$</th>
<th>$Hg(ClO_4)_2$ $F \times 10^4$</th>
<th>Purine or Pyrimidine $F \times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.10</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Electrolyte: $\sim 6F NaCl$ and $0.6F HCl$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compartments K and A contain electrolyte. From K to inlet tube E is the 36% $D_2O$ solution (no. 1). The 8–28% $D_2O$ density gradient containing the boundary and obtained from solutions 2–5 extends from E to about junction B. The position of the boundary is approximately indicated in Figure 3. Above the gradient are 4 ml of soln. 6 and between soln. 6 and the top electrolyte is soln. 7.
The electrolyte (no. 8) is saturated NaCl. It is doped with 0.6 M HCl in an attempt to keep the ratio of $H^+$/Na$^+$ ions leaving the electrode compartment of the same magnitude as that in the rest of the solutions.

In unbuffered acid solutions positive ions are moving toward the nearest electrode compartment so that there is no possibility that the fast moving $H^+$ ion is being supplied in too large or too small amounts from the electrode compartment thus leading to pH changes at the electrophoresis boundary.

In solutions 1 to 7 0.10 M NaClO$_4$ has been chosen as the supporting electrolyte. The purine concentration in solutions 3 and 5 has been picked so that the absorbance is in the range of 1.0-1.2 giving best accuracy. For the light intensity (I$_o$) that has been obtained a solution having an absorbance less than 0.1 does not absorb enough light to give accurate results while above absorbances of 2 essentially no light reaches the phototube.

The necessary $H^+$ and Hg$^{++}$ ion concentrations for solutions 1 to 7 are then calculated from the known purine (or pyrimidine) concentration and the K's that were obtained by PEXYVD. Solutions 1 to 6 have identical compositions except for the absorbing material so that as current is passed and the boundary moves there will be no net change in the environment in the neighborhood of the boundary.

Solution 7 is chosen so that the ions leaving it do not react with the electrolyte or at the electrode in such a way as to change the conditions in the optical part of the cell. Thus solution 7 of Table 2 has no Hg$^{++}$ ions in it since it was found that these ions reach the top electrode forming Hg$_2$Cl$_2$ and making the electrode irreversible. If the current is passed long enough solid Hg$_2$Cl$_2$ starts falling down the cell. Another example would be the reaction between Cl$^-$ and CH$_3$Hg-OH liberating OH$^-$ and
thus changing the pH within a matter of minutes.

The D$_2$O concentrations of solutions 2-5 lead to a linear gradient varying between 8-28%. The discontinuities in density between the gradient and solutions 1 and 6 lead to better layering and less disturbance when the gradient is passed in between 1 and 6.

The H$^+$ concentrations of solutions 1 to 7 were checked before the filling of the cell with a Beckman GS type pH meter. Also the spectra of solutions 3 and 5 were taken. All spectra have been measured with a Cary Model 14 spectrophotometer. Occasionally the conductivities of solns. 1 to 7 were checked by the use of a Kohlrausch cell. KCl solution was used to determine the cell constant.

For a number of experiments after mobility measurements had been made all the solution in the electrophoresis cell was removed in 1 ml portions and both spectra and pH's were measured to determine whether initial conditions had changed.

Whenever solutions 3 and 5 contained Hg$^{+2}$ or CH$_3$Hg$^+$ complexes the filling of the cell and all succeeding steps were carried out in the presence of dim tungsten lights. The light path is blocked except when taking a measurement.

c. Electrophoresis

Curve I$_b$ of Figure 4 is the base line obtained by blocking the light path in front of window h (Fig. 1). The curve marked I$_o$ is the initial intensity measured just before passing the density gradient into the cell. The sharp drops in intensity are due to the wire markers in front of the cell.
Fig 4

7-Methyladenine; $H^+$

+ ions

$(I_b)$ - base line is parallel to the x-axis at 0 intensity units
Curve 1 represents the initial boundary having an ~ 6 mm spread. The boundary can be sharpened by careful removal of solution between points x and y. Curve 2 is the result. The disadvantage of this procedure is that some of the density gradient is lost and a slight schlieren effect is obtained so that for meaningful measurements the boundary has to be moved away quickly from the point of sharpening.

The power supply is then turned on and is set at a voltage that gives a 7 to 15 mA current. The voltage drop in the optical part of the cell varies between 1 and 2 v/cm. Under these conditions not enough heat is liberated to set up convection and at the same time the boundaries do move at reasonable speeds. The possibility of convection is kept to a minimum by doing the experiments at 6.0°C which is close to the temperature of maximum density for the solutions used. Actual measurement of the temperature with the thermocouple probe showed no detectable change in temperature.

Enough intensity versus distance curves are taken so that the constancy of the mobility can be checked and any large deviations from expected behavior observed.

It is assumed that $I_0$ remains constant during an experiment. If the lamp intensity varies, this is compensated by the gain control until $I_0$ is reproduced. Because of this the experiments are discontinued when one cannot observe $I_0$ directly at some point in the cell (point Z in Fig. 4). The mobility can be measured in from 2 to 5 hours while diffusion experiments take between 2 to 6 hours. Curves 3 to 4 in Figure 2 show a two hour diffusion run in the absence of an electric field.
IV. CALCULATIONS AND RESULTS FOR ELECTROPHORESIS AND DIFFUSION

The curves obtained in Figure 4 are exponential since the intensity $I$ is given by Beer's Law

$$I = I_0 e^{-ck}$$

where

- $I_0$ = initial intensity
- $I$ = intensity
- $k$ = constant
- $c$ = concentration of absorbing material

The type of plot that is most useful is that of $\frac{c_x}{c_m}$ versus $x$ where $c_x$ is the concentration of the absorbing species at any position $x$ in the cell and $c_m$ is the maximum or initial concentration.

Using equation (10) one obtains the following relationship

$$\frac{c_x}{c_m} = \frac{\ln \frac{I_x}{I_0}}{\ln \frac{I_{mc}}{I_0}}$$

where

- $I_x$ = intensity at any position $x$
- $I_0$ = initial intensity at position $x$
- $I_{mc}$ = intensity at position $x$ if the maximum concentration of absorbing material were there.

All of the above quantities can and are obtained from the experimental curves, so that $\frac{c_x}{c_m}$ can be easily calculated. Figure 5 shows the concentration profiles of Figure 4. The distance travelled by a boundary can be measured directly by measuring the displacement at $c = \frac{1}{2} c_m$ since the motion here is unaffected by diffusion as seen in the next paragraph.
Fig 5
Concentration Profiles of
(7-Methyladenine-H)^+

$\frac{C_X}{C_m}$ vs DISTANCE IN MM

+ ions
For the type of boundary that has been used in these experiments

\[ c(r,t) = \frac{1}{2} c_m \left( 1 - \text{erf} \left( \frac{r}{2\sqrt{D}t} \right) \right) \]

so that \( r \) indeed remains 0 at \( c(r,t) = \frac{1}{2} c_m \). Also if the experiment is behaving properly and if the initial boundary is not skew then all the concentration curves should be symmetric about \( \frac{c_x}{c_m} = 0.5 \).

Since \( r \) and \( t \) are measurable quantities and error functions can be obtained from tables (5) the diffusion constants can be calculated. This has been done for a number of cases (see tables 4-11) both for moving (\( m \)) and stationary (\( s \)) boundaries. The listed values of \( D \) are for \( \frac{c_x}{c_m} = 0.3 \) because best reproducibility is obtained in this region.

The mobilities \( u \) were calculated by the use of the formula

\[ u = \frac{\Delta x}{\Delta t E} \]

\( \Delta x \) the distance that the boundary moves and \( \Delta t \) the length of time the field is on were measured for each experiment. The electric field \( E \) was calculated from the conductances of the ionic constituents of the supporting electrolyte.
(6,7) (corrected approximately to the ionic strength of the experiments) with the following formula,

\[
E = \frac{1}{\sum_j \Lambda_j c_j A}
\]

\(i = \text{current}\)
\(\Lambda_j = \text{conductance of ion } j\)
\(c_j = \text{concentration of ion } j\)
\(A = \text{cross section of cell} = 1 \text{ cm}^2\)  

The current is measured with an accuracy of about 0.5%. Table 3 gives the values of \(E\) as a function of \(i\) for the different experiments. The \(\Lambda\)'s used in the calculation of \(E\) were checked by actual measurement of the conductivities at 6.0°C. Also in a number of cases the actual voltage drop in the cell was measured giving another check. The \(E\) values should therefore be good to one or two per cent. If better \(\Lambda\) values are obtained then Table 3 allows to correct the calculated mobilities.

Tables 4-11 inclusive list the results of the mobility and diffusion experiments. Additional information about experimental conditions is listed below each table.
TABLE 3

\[ E = k \times i \]
\[ \frac{V}{cm} \text{ amp} \]

<table>
<thead>
<tr>
<th>( k )</th>
<th>Expt. no.'s</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.3</td>
<td>36</td>
</tr>
<tr>
<td>75.9</td>
<td>2, 3, 22, 23, 24, 28, 30, 34</td>
</tr>
<tr>
<td>126</td>
<td>37, 38</td>
</tr>
<tr>
<td>132</td>
<td>9</td>
</tr>
<tr>
<td>136</td>
<td>16, 17, 18, 25, 26, 27, 29, 32, 33, 35</td>
</tr>
<tr>
<td>141</td>
<td>19</td>
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<tr>
<td>154</td>
<td>4, 7</td>
</tr>
<tr>
<td>163</td>
<td>20</td>
</tr>
<tr>
<td>166</td>
<td>5, 6</td>
</tr>
<tr>
<td>167</td>
<td>21, 39, 40</td>
</tr>
<tr>
<td>177</td>
<td>15</td>
</tr>
<tr>
<td>208</td>
<td>1</td>
</tr>
</tbody>
</table>

Conductivity measured for 4, 7, 9, 19.

\[ \frac{V}{cm} \] measured directly for 0, 1, 10, 11, 12, 14.
### Table 4

Adenine

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material $\times 10^5$</th>
<th>$T$ °C</th>
<th>Gradient</th>
<th>pH (25°C)</th>
<th>$u \times 10^4$</th>
<th>$D_s \times 10^6$</th>
<th>$D_m \times 10^9$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>adenine; $H^+$</td>
<td>4.89</td>
<td>0.0 ± 0.1</td>
<td>sucrose</td>
<td>2.14</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>adenine; $H^+$</td>
<td>4.89</td>
<td>0.0 ± 0.1</td>
<td>sucrose</td>
<td>2.14</td>
<td>1.09</td>
<td>2.1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>adenine; $H^+$</td>
<td>4.89</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>1.60</td>
<td>1.77</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>adenine; $Hg^{+2}$</td>
<td>7.47</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>1.60</td>
<td>2.14</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>adenine; $CH_3Hg^+$</td>
<td>7.47</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>4.73</td>
<td>1.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>adenine; $CH_3Hg^+$</td>
<td>7.47</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>4.73</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>adenine; $CH_3Hg^+$</td>
<td>7.47</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>4.73</td>
<td>1.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>adenine; $CH_3Hg^+$</td>
<td>9.96</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>3.00</td>
<td>1.18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>adenine; $CH_3Hg^+$</td>
<td>9.96</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>3.50</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>adenine; $CH_3Hg^+$</td>
<td>9.96</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>5.90</td>
<td>0.749</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$\Sigma$ conc. (F) .06 .0073 .10 .025 3.0 x $10^{-6}$ 8.12 x $10^{-3}$ 1.01 x $10^{-2}$ 5.22 x $10^{-2}$

| $\Sigma$ conc. (P) | 0.1 | 0.1 |
| $\Sigma$ conc. (H) | 0.25 |
| $\Sigma$ conc. (OH) | 2.9 |

pH of solutions 3-9 adjusted by addition of appropriate amounts of concentrated $HCl$ or $NaOH$.

Solution 4 also had .0035 M $NaAc$ and .0025 M $HAc$.

Solution 9 also had .057 $NaAc$ and .003 M $HAc$. 
<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material x $10^5$</th>
<th>$T$ °C</th>
<th>Gradient</th>
<th>pH (23°C)</th>
<th>$u \times 10^2$</th>
<th>$D_a \times 10^6$</th>
<th>$D_{ba} \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>0.0 ± 0.1</td>
<td>sucrose</td>
<td>2.14</td>
<td>0.777</td>
<td>1.4</td>
<td>-</td>
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<tr>
<td>11</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>6.0 ± 1.0</td>
<td>sucrose</td>
<td>2.14</td>
<td>0.905</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>8.0 ± 0.3</td>
<td>sucrose</td>
<td>~ 7</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>8.0 ± 0.6</td>
<td>sucrose</td>
<td>2.14</td>
<td>-</td>
<td>-</td>
<td>3.2</td>
</tr>
<tr>
<td>14</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>10.0 ± 0.5</td>
<td>sucrose</td>
<td>2.14</td>
<td>1.14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>adenosine; $H^+$</td>
<td>7.98</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.14</td>
<td>1.12</td>
<td>-</td>
<td>2.1</td>
</tr>
<tr>
<td>16</td>
<td>adenosine; $Hg^{42+}$</td>
<td>7.98</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.30</td>
<td>0.744</td>
<td>-</td>
<td>2.4</td>
</tr>
<tr>
<td>17</td>
<td>adenosine; $Hg^{42+}$</td>
<td>7.98</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.30</td>
<td>0.762</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>adenosine; $Hg^{42+}$</td>
<td>7.98</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.30</td>
<td>0.727</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>adenosine; $\text{CH}_3-Hg^+$</td>
<td>8.82</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>4.20</td>
<td>0.585</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>conc. (F)</th>
<th>.06</th>
<th>.0073</th>
<th>0.10</th>
<th>8.0 x $10^{-4}$</th>
<th>5.67 x $10^{-2}$</th>
</tr>
</thead>
</table>

| $\text{NaCl}$ | 10-14 | .0073          | 0.10  | 8.0 x $10^{-4}$ | 5.67 x $10^{-2}$ |
| $\text{HCl}$  |       |                 |       |                |                |
| $\text{NaClO}_4$ | 15   | 10,11,13,14| 16-19 |                |                |
| $\text{HClO}_4$ |       | 15             |       |                |                |
| $\Sigma \text{Hg}$ | 15   | 16-18          |       |                |                |
| $\Sigma \text{CH}_3-Hg$ | 19   |                |       |                |                |

pH of solutions 12, 16-19 adjusted by addition of appropriate amounts of $\text{HClO}_4$ or NaOH.
### TABLE 6

Deoxyadenylic Acid (5')

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material $\times 10^5$</th>
<th>$T$ $^\circ$C</th>
<th>Gradient</th>
<th>pH</th>
<th>$u \times 10^6$</th>
<th>$D_s \times 10^6$</th>
<th>$D_m \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>deoxyadenylic acid (5'); $H^+$</td>
<td>8.46</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>5.00</td>
<td>0.813</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>21</td>
<td>deoxyadenylic acid (5'); $H^+$</td>
<td>8.46</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>8.06</td>
<td>1.30</td>
<td>2.2</td>
<td>2.6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Conc. (F)</th>
<th>0.10</th>
<th>0.0043</th>
<th>0.0017</th>
<th>0.006</th>
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<tbody>
<tr>
<td>$NaClO_4$</td>
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<td></td>
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<tr>
<td>$NaAc$</td>
<td>20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\Pi Ac$</td>
<td>20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\Pi Tris$ buffer</td>
<td>20</td>
<td></td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Expt. no.</td>
<td>Reactants of interest</td>
<td>$\Sigma$ conc. of absorbing material $\times 10^5$</td>
<td>$T$ $^\circ C$</td>
<td>Gradient</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>22</td>
<td>7-methyladenine; $H^+$</td>
<td>6.64</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
</tr>
<tr>
<td>23</td>
<td>7-methyladenine; $Hg^{+2}$</td>
<td>6.48</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
</tr>
<tr>
<td>24</td>
<td>7-methyladenine; $Hg^{+2}$</td>
<td>6.48</td>
<td>7.0 ± 0.1</td>
<td>$D_2O$</td>
</tr>
<tr>
<td>25</td>
<td>7-methyladenine; $Hg^{+2}$</td>
<td>6.48</td>
<td>6.2 ± 0.1</td>
<td>$D_2O$</td>
</tr>
</tbody>
</table>

$\Sigma$ conc. $(F) = 0.10$; $0.025$; $8.0 \times 10^{-4}$

$pH$ of solutions 23–25 adjusted by addition of appropriate amounts of $HCIO_4$.

solution 23–electrophoresis 7 days after mixing 7-methyladenine and $Hg^{+2}$ ion.

24— 10 hrs.  
25— immediately after mixing 7-methyladenine and $Hg^{+2}$ ion.
<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material x $10^5$</th>
<th>T (°C)</th>
<th>Gradient</th>
<th>pH (25° C)</th>
<th>$u \times 10^4$</th>
<th>$D_s \times 10^6$</th>
<th>$D_m \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>9-Methyladenine; H⁺</td>
<td>8.15</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.30</td>
<td>1.53</td>
<td>3.4</td>
<td>5.2</td>
</tr>
<tr>
<td>27</td>
<td>9-Methyladenine; $\Pi C^+$</td>
<td>8.15</td>
<td>6.0 ± 0.1</td>
<td>$D_2O$</td>
<td>2.30</td>
<td>1.45</td>
<td>2.7</td>
<td>3.8</td>
</tr>
</tbody>
</table>

**Table 8: 9-Methyladenine**

<table>
<thead>
<tr>
<th>conc. (f)</th>
<th>0.10</th>
<th>0.005</th>
<th>$8.0 \times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaClO₄</td>
<td>26, 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HClO₄</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Sigma$ $\Pi C$</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pH of solution 27 adjusted by addition of conc. HClO₄.
<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material x $10^5$</th>
<th>$T$ °C</th>
<th>Gradient</th>
<th>pH (23° C)</th>
<th>$u \times 10^4$</th>
<th>$D_s \times 10^6$</th>
<th>$D_m \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>6-N,N-dimethyl-aminopurine; H⁺</td>
<td>6.42</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>1.60</td>
<td>1.27</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>29</td>
<td>6-N,N-dimethyl-aminopurine; H⁺</td>
<td>6.42</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>2.30</td>
<td>1.33</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>6-N,N-dimethyl-42-aminopurine; H⁺</td>
<td>6.42</td>
<td>7.0 ± 0.1</td>
<td>D₂O</td>
<td>1.60</td>
<td>1.62</td>
<td>2.1</td>
<td>2.8</td>
</tr>
<tr>
<td>31</td>
<td>6-N,N-dimethyl-42-aminopurine; H⁺</td>
<td>6.42</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>2.30</td>
<td>-</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td>6-N,N-dimethyl-42-aminopurine; H⁺</td>
<td>6.42</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>2.30</td>
<td>1.40</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>33</td>
<td>6-N,N-dimethyl-42-aminopurine; H⁺</td>
<td>6.42</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>2.30</td>
<td>1.40</td>
<td>-</td>
<td>2.3</td>
</tr>
</tbody>
</table>

For conc. (F) 0.10 0.025 0.005 $3.0 \times 10^{-4}$

- Na₂SO₄ 23-33
- HClO₄ 28 29
- $\Sigma$ Hg 30-33

Ph of solutions 30-33 adjusted by addition of conc. HClO₄.
### TABLE 10

<table>
<thead>
<tr>
<th>Expt. Reactants of interest</th>
<th>$\Sigma$ conc. of absorbing material x 10$^5$</th>
<th>$T^\circ$ C</th>
<th>Gradient</th>
<th>pH (25$^\circ$ C)</th>
<th>$u \times 10^4$</th>
<th>$D_s \times 10^6$</th>
<th>$D_m \times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>cytidine; H$^+$</td>
<td>8.40</td>
<td>6.0 ± 0.1</td>
<td>D$_2$O</td>
<td>1.60</td>
<td>0.980</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>cytidine; H$^+$</td>
<td>9.38</td>
<td>6.0 ± 0.1</td>
<td>D$_2$O</td>
<td>2.30</td>
<td>1.00</td>
<td>2.3</td>
</tr>
<tr>
<td>36</td>
<td>cytidine; Hg$^{+2}$</td>
<td>9.88</td>
<td>6.0 ± 0.1</td>
<td>D$_2$O</td>
<td>1.60</td>
<td>1.50</td>
<td>-</td>
</tr>
<tr>
<td>37</td>
<td>cytidine; Hg$^{+2}$</td>
<td>9.38</td>
<td>6.0 ± 0.1</td>
<td>D$_2$O</td>
<td>2.30</td>
<td>1.03</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td>cytidine; Hg$^{+2}$</td>
<td>9.88</td>
<td>6.0 ± 0.1</td>
<td>D$_2$O</td>
<td>~2.30</td>
<td>1.05</td>
<td>-</td>
</tr>
<tr>
<td>conc. (F)</td>
<td>0.10</td>
<td>0.025</td>
<td>0.005</td>
<td>8.0 x 10$^{-3}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| NaClO$_4$ | 34-38 | | |
| HClO$_4$ | 34-38 |
| $\Sigma$ Hg | 34 | 35 | 36-38 |

pH of solutions 36-38 adjusted by addition of conc. HClO$_4$. 
## TABLE 11

**DNA (calf thymus)**

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Reactants of interest</th>
<th>Σ conc. of absorbing material x 10^5</th>
<th>T °C</th>
<th>Gradient</th>
<th>pH (6°C)</th>
<th>cm/sec volt/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>DNA (calf thymus)</td>
<td>absorbance of ~ 1.3</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>3.0</td>
<td>1.39</td>
</tr>
<tr>
<td>40</td>
<td>DNA (calf thymus)</td>
<td>heat denatured</td>
<td>6.0 ± 0.1</td>
<td>D₂O</td>
<td>3.0</td>
<td>1.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>conc. (F)</th>
<th>0.10</th>
<th>.006</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaClO₄</td>
<td>39,40</td>
<td></td>
</tr>
<tr>
<td>Σ Tris buffer</td>
<td>39,40</td>
<td></td>
</tr>
</tbody>
</table>

*by heating at 100° for 30 minutes.*
V. SPECTROPHOTOMETRIC AND ELECTROPHORETIC STUDY
OF THE METHYL MERCURY-ADENINE COMPLEXES

Figure 6a shows the spectra of a set of solutions containing
9.96 x 10^{-5} F adenine and 1.74 x 10^{-2} F CH_3-Hg^+ as a function of pH.
CH_3-Hg^+ and CH_3-Hg-OH do not absorb strongly until \( \lambda = 230 \) nm so the
main features of the spectra in Figure 6a are due to adenine and its com-
plexes. Clearly there are several absorbing species.

If the formation and dissociation of the complexes takes place in
a stepwise manner, so that only one reaction is taking place to a signifi-
cant extent in a given range of concentrations, the analysis of the data
for a reaction such as

\[
P-(\text{HgCH}_3)_x^{+n} + m \text{ Hg-C}_{3}H_{5}^{+} \rightleftharpoons P-(\text{HgCH}_3)_x^{+(n+m-x)} + xH^+ \]

can be made using the equation

\[
\frac{1}{e - \epsilon_0} = \frac{1}{\epsilon_1 - \epsilon_0} + \frac{1}{(\epsilon_1 - \epsilon_0)K_{\text{HgCH}_3^{+}}} \frac{(H^+)_x^{m}}{(H_2)} \tag{15}
\]

\( \epsilon_0 \) and \( \epsilon_1 \) are the extinction coefficients of \( P-(\text{HgCH}_3)_x^{+n} \) and \( P-(\text{HgCH}_3)_x^{+(n+m-x)} \)
respectively. \( \bar{e} \) is the formal extinction coefficient per mole of ligand.

A plot of the left side of equation (15) against \( \frac{(H^+)_x^{m}}{(H_2)} \) should
give a straight line if the correct \( x \) and \( m \) are chosen. \( K \) and \( \epsilon_1 \) can then
be calculated from the slope and the intercept. Figure 7 shows a typical
plot for the reaction

\[
\begin{align*}
\begin{array}{c}
\text{[N]} \\
\text{H} \\
\text{N} \\
\text{H}
\end{array} \\
\begin{array}{c}
\text{[N]} \\
\text{H}
\end{array} \\
\begin{array}{c}
\text{H}
\end{array}
\rightleftharpoons
\begin{array}{c}
\text{[N]} \\
\text{H}
\end{array} + \text{H}^+
\end{align*}
\tag{16}
\]
Fig 6

\[ \Sigma \text{CH}_3\text{-Hg}^+ = 1.74 \times 10^{-2} \text{F} \]
\[ \Sigma A = 9.96 \times 10^{-5} \text{F} \]
\[ \text{pH} = 0.80 - 12.50 \]

\[ \Sigma A = 9.96 \times 10^{-5} \]
Fig 7

Typical Plot of Equation (15) for Reaction (16)

\[
\frac{e - e_0}{e_0} = 10^{4} \times \sum A
\]

\[
\sum \text{CH}_3\text{Hg} = 8.12 \times 10^{-3}
\]

\[
\sum \text{CH}_3\text{Hg} = 2.67 \times 10^{-3}
\]

\[
\text{pH} = 1.37 - 2.34
\]

\[
\text{pH} = 2.14 - 2.94
\]
Using the above technique three different adenine-methyl mercury complexes were identified. Table 12 lists the reactions involved and the equilibrium constants. Also listed are the constants for some other reactions obtained in the course of this investigation.

Figure 6b shows the spectra of (adenine H)⁺, the three methyl mercury complexes IV, V, and VI, and of (adenine)⁻. The spectral changes taking place in Figure 6a should be then easily identifiable. Finally Figure 8 shows the relative concentrations of the five adenine species as a function of pH for the case when $\Sigma$ CH₃-Hg is $10^{-2}$ F.

The electrophoretic mobilities under various conditions are listed in Table 13. Analysis of the data leads to the following mobilities for the different adenine complexes,

\[
\begin{align*}
[\text{Adenine H}]^+ & \quad - 1.77 \pm .02 \times 10^{-4} \\
[\text{Adenine-HgCH}_3]^+ & \quad - 1.25 \pm .09 \times 10^{-4} \\
[\text{Adenine-}(\text{HgCH}_3)_2]^+ & \quad - 0.74 \pm .10 \times 10^{-4} \\
[\text{Adenine-}(\text{HgCH}_3)_2]^0 & \quad - 0.00 \pm .02 \times 10^{-4}
\end{align*}
\]

Thus we note that replacing a proton in adenine with a CH₃-Hg⁺ decreases the mobility by 29%; replacing a second proton by CH₃-Hg⁺ decreases the mobility by an additional 29%.

It is perhaps not inappropriate to remark that the spectrophotometric investigation of the methyl mercury complexes was undertaken after a measurement of the electrical mobility of $u = 1.00 \times 10^{-4}$ was obtained at pH 4.73 and $\Sigma$ CH₃-Hg = 8.12 x $10^{-3}$. It was assumed that under these conditions the complex present was [adenine-HgCH₃]⁺, but the change in mobility from [adenine-H]⁺ seemed too large by comparison with the results of the
Hg$^{+2}$ complexes, and by comparison with the difference between protonated adenine and adenosine. Thus, in this case, electrophoretic methods have proved their usefulness as a semiquantitative clue to structural features.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>$K(27^\circ C)(\mu=0.1)$</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Reactor} A^+ + \text{CH}_3\text{Hg}^+ \rightleftharpoons \text{IV} + \text{H}^+$</td>
<td>3.6</td>
<td>spectrophotometric</td>
</tr>
<tr>
<td>$\text{IV} + \text{CH}_3\text{Hg}^+ \rightleftharpoons \text{V} + \text{H}^+$</td>
<td>$4.84 \times 10^{-3}$</td>
<td>spectrophotometric</td>
</tr>
<tr>
<td>$\text{V} + \text{OH}^- \rightleftharpoons \text{VI} + \text{CH}_3\text{Hg-OH}$</td>
<td>$2.3 \times 10^4$</td>
<td>spectrophotometric</td>
</tr>
<tr>
<td>$\text{VI} + \text{OH}^- \rightleftharpoons \text{V} + \text{CH}_3\text{Hg-OH}$</td>
<td>1.8</td>
<td>spectrophotometric</td>
</tr>
</tbody>
</table>
IV + OH$^- \rightleftharpoons$ VI + H$_2$O

\[ \text{CH}_3\text{-Hg}^+ + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{-Hg-OH} + \text{H}^+ \]

3.5 x 10$^6$ from K's listed here

2.76 x 10$^{-5}$ pH-titration

3.16 x 10$^{-4}$ Cal. Biochem. Corp. Tables

1.58 x 10$^{-10}$ spectrophotometric

2.44 x 10$^{-3}$ pH-stat
Fig 8

Relative Concentrations of (Adenine-H)$^+$, Complexes IV, V, VI and (Adenine)$^-$ as a Function of the Acidity

$\Sigma \text{CH}_3\text{Hg} = 1.0 \times 10^{-2} F$
### TABLE 13

Electrophoretic Mobilities of Methyl Mercury-
Adenine Under Various Conditions

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Σ CH₃-Hg</th>
<th>Σ A x 10⁵</th>
<th>pH</th>
<th>A⁺</th>
<th>IV⁺</th>
<th>V⁺</th>
<th>VI⁰</th>
<th>A⁻</th>
<th>u x 10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8.12 x 10⁻³</td>
<td>7.47</td>
<td>4.73</td>
<td>-</td>
<td>53.8</td>
<td>46.2</td>
<td>-</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>5</td>
<td>8.12 x 10⁻³</td>
<td>7.47</td>
<td>4.73</td>
<td>-</td>
<td>53.8</td>
<td>46.2</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>8.12 x 10⁻³</td>
<td>7.47</td>
<td>4.73</td>
<td>-</td>
<td>53.8</td>
<td>46.2</td>
<td>-</td>
<td>-</td>
<td>1.05</td>
</tr>
<tr>
<td>7</td>
<td>1.01 x 10⁻²</td>
<td>9.96</td>
<td>3.00</td>
<td>2.6</td>
<td>92.9</td>
<td>4.5</td>
<td>-</td>
<td>-</td>
<td>1.18</td>
</tr>
<tr>
<td>8</td>
<td>1.01 x 10⁻²</td>
<td>9.96</td>
<td>9.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>~100</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>5.22 x 10⁻²</td>
<td>9.96</td>
<td>5.90</td>
<td>-</td>
<td>10.3</td>
<td>89.7</td>
<td>-</td>
<td>-</td>
<td>0.749</td>
</tr>
</tbody>
</table>
VI. A CRITICAL REVIEW OF THE FELDYD SPECTROPHOTOMETRIC
INVESTIGATION OF THE PURINE-MERCURIC ION SYSTEMS

The mercuric ion-adenosine system has been investigated more
extensively than any other.

Figure 9 shows the spectral changes as a function of pH for a
solution containing $4.0 \times 10^{-5}$ F adenosine and $1.6 \times 10^{-3}$ F mercuric ion.
Curve 1 gives the spectrum of protonated adenosine in the absence of $\text{Hg}^{+2}$
ion. As the pH is increased first one complex forms and then as shown
by curve 5 another reaction occurs. Thus first of all one is almost
always limited in all the investigations to small concentration ranges
with of course a corresponding decrease in the reliability of the method
used.

Further complications that should be kept in mind are that the
spectral changes are relatively small and that with the type of plots
used one has to apply a correction to the $\text{Hg}^{+2}$ concentration due to the
formation of $\text{Hg-OH}^+$ and $\text{Hg(OH)}_2^-$.

Figure 10 shows the type of plots already discussed for the follow-
ing reactions,

$$
\begin{align*}
\left[ \begin{array}{c}
\text{NH}_2 \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{R} \\
\text{N} \\
\text{R}
\end{array} \right] + \text{Hg}^{+2} + 2 \text{H}^+ & \rightleftharpoons \left[ \begin{array}{c}
\text{NH}_2 \\
\text{H} \text{OH}_2 \\
\text{N} \\
\text{N} \\
\text{R} \\
\text{N} \\
\text{R}
\end{array} \right]

tag{17}
\end{align*}
$$

$$
\begin{align*}
\left[ \begin{array}{c}
\text{NH}_2 \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{R} \\
\text{N} \\
\text{R}
\end{array} \right] + \text{Hg}^{+2} & \rightleftharpoons \left[ \begin{array}{c}
\text{NH}_2 \\
\text{H} \text{OH} \\
\text{N} \\
\text{N} \\
\text{R} \\
\text{N} \\
\text{R}
\end{array} \right] + \text{H}^+

tag{18}
\end{align*}
$$
Fig 9
Spectra of Mercury(II) Adenosine Complex

<table>
<thead>
<tr>
<th>As</th>
<th>[Hg^{+2}]</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0 x 10^{-5}</td>
<td>2.86</td>
</tr>
<tr>
<td>2</td>
<td>1.6 x 10^{-3}</td>
<td>2.05</td>
</tr>
<tr>
<td>3</td>
<td>2.24</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.42</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.84</td>
<td></td>
</tr>
</tbody>
</table>
Fig 10a

\[ \sum \text{Hg}^{+2} F \]

\[ \lambda = 255 \text{ m}\mu \]

\[ \lambda = 270 \text{ m}\mu \]

\[ \lambda = 290 \text{ m}\mu \]

\[ 4.00 \times 10^{-4} \quad 2.41 - 2.78 \]

\[ 1.60 \times 10^{-3} \quad 2.05 - 2.42 \]

\[ \sum \text{Adenosine} = 3.99 \times 10^{-5} \text{ F} \]

\[ \frac{(H^+)^2}{(Hg^{+2}) \times 10^3} \]

Fig 10b

\[ \frac{(H^+)^2}{(Hg^{+2})} \times 10^3 \]

\[ \lambda = 270 \text{ m}\mu \]

\[ \sum \text{Hg}^{+2} F \]

\[ \text{pH} \]

\[ 4.00 \times 10^{-4} \quad 2.41 - 2.78 \]

\[ 1.60 \times 10^{-3} \quad 2.05 - 2.42 \]

\[ \sum \text{Adenosine} = 3.99 \times 10^{-5} \text{ F} \]
Figure 10a shows a plot of \( \frac{1}{e-e_0} \) versus \( \frac{(H^+)^2}{(\text{Hg}^{2+})} \) for a range of pH's and mercuric ion concentrations at three different wavelengths. One obtains essentially straight lines with the same K. Figure 10b shows the plot at \( \lambda = 270 \, \text{nm} \) for reaction 18. It is interesting to note that the points at different pH's but the same total mercuric ion concentration lie on approximately a straight line. This is no longer true when the mercuric ion concentration is changed. Further experiments also show that there is a certain amount of curvature for the plots for reaction 18 and the same total \( \text{Hg}^{2+} \) concentration. The results seem to indicate with a high degree of certainty that reaction 17 is taking place rather than 18. The nature of the complex found at lower values of \( H^+/\text{Hg}^{2+} \) (curve 5) is not known. It would be difficult to investigate because of the hydrolysis of \( \text{Hg}^{2+} \).

The 9-methyladenine-\( \text{Hg}^{2+} \) system results are similar to adenosine but not quite as convincing. A straight line plot is obtained for the release of 2 protons as a variation of pH and \( \Sigma \text{Hg}^{2+} \) while some scattering of points is obtained for the release of one proton (see Fig. 11a and b). Again only a limited range of concentrations of \( H^+ \) and \( \text{Hg}^{2+} \) can be looked at because of further different spectral changes.

The adenine-\( \text{Hg}^{2+} \) system shows different behavior. Even though both the \( \Sigma \text{Hg}^{2+} \) and \( H^+ \) concentrations are varied a reasonably straight curve is obtained in both cases indicating some sort of complication in the reactions taking place. Figure 12 shows a set of curves and should be compared to Figures 10 and 11. Quite possibly both reactions

\[
\text{[adenine-H]}^+ + \text{Hg-OR}_2^{2+} \rightleftharpoons \text{[adenine-Hg-OR}_2]^+ + H^+
\]

\[
\text{[adenine-Hg-OR}_2]^+ \rightleftharpoons \text{[adenine-Hg-OH]}^+ + H^+
\]
Fig Ila

\[ \frac{1}{\left( \frac{\Sigma Hg^{+2} F}{Hg^{+2}} \right) \times 10^3} \]

\[ \lambda = 280 \]

\[ \Sigma Hg^{+2} F \]

- 1.50 x 10^{-3} 1.98 - 2.65
- 2.0-5.0 x 10^{-3} 2.00

\[ \Sigma 9\text{-Methyladenine} = 1.07 \times 10^{-4} F \]

Fig IIb

\[ \frac{1}{\left( \frac{H^+}{Hg^{+2}} \right) \times 10^4} \]

\[ \lambda = 280 \]

\[ \Sigma Hg^{+2} F \]

- 1.50 x 10^{-3} 1.98 - 2.65
- 2.0-5.0 x 10^{-3} 2.00

\[ \Sigma 9\text{-Methyladenine} = 1.07 \times 10^{-4} F \]
Fig 12a

\[ \lambda = 280 \text{ nm} \]

\[ \frac{1}{[H^+]} \times 10^4 \]

\[ \frac{1}{[Hg^{2+}]} \]

\[ \text{pH} = 1.31 - 2.09 \]
\[ \Sigma Hg^{2+} = 1.0 - 2.0 \times 10^{-3} \text{ F} \]
\[ \Sigma \text{Adenine} = 8 \times 10^{-5} \text{ F} \]

Fig 12b

\[ \lambda = 280 \text{ nm} \]

\[ \frac{[H^+]}{[Hg^{2+}]} \]

\[ \frac{[H^+]}{[Hg^{2+}]} \times 10^4 \]
are taking place. It is also conceivable, since adenine has an additional N-H group, that a dimercurated species forms. A precipitate forms if the pH of a Hg$^{2+}$-adenine solution is lowered below 2. Thus it is difficult to study this system over a wide range of concentrations.

The 6-N,N-dimethylaminopurine system has been investigated only as a function of pH. Both the plots for 1 and 2 proton liberation seem to give straight lines. However as has been shown for the above 3 cases without substantial Hg$^{2+}$ variation no definite conclusion can be drawn as to which reaction is taking place.

A careful look at the spectrophotometric investigations done thus far indicate then that for adenosine and 9-methyladenine in all probability the following reaction is taking place,

$$\text{HP}^+ + \text{Hg-} \text{OH}^+ \rightleftharpoons \text{P-Hg-OH}^+ + 2\text{H}^+$$

(19)

while in the case of adenine the reaction is probably more complex. Also there is not enough information to determine the possible reaction for 6-N,N-dimethylaminopurine.
VII. CHARGES AS DETERMINED BY COMPARISON OF THE ELECTROPHORETIC VELOCITIES OF STRUCTURALLY SIMILAR MOLECULES

Table 1 lists the mobilities of the various purine and pyrimidine systems at 6.0°C and in the presence of a D2O gradient. The left side of the table gives a comparison of the mobilities of the protonated systems of known charge and the right side shows the observed changes in the electrophoretic velocities when \( \text{Hg}^{2+} \) or \( \text{CH}_3\text{Hg}^+ \) ions are present under similar conditions. For comparison purposes the mobilities of the adenine-methylmercury complexes are also listed.

In the first place it is observed that adenylic acid at pH 5 with a charge of -1 has a mobility of \( 0.81 \times 10^{-4} \) while at pH 8 with a charge of -2 the mobility is \( 1.30 \times 10^{-4} \). Thus when the charge increases by a factor of two the mobility increases by a factor of 1.6. The extra drag is presumably due to increased solvation around the binegative phosphate group, on which the charge is localized.

As shown by equation 5 the mobility is inversely proportional to the friction coefficient \( f \). For a spherical molecule \( f \) is proportional to the one-third power of the molecular volume. Qualitatively speaking, this would suggest that the mobilities would decrease as methyl or other bulky groups are added to adenine. The addition of methyl groups would also change the solvation directly and perhaps indirectly by changing the charge distribution on the ions and these phenomena too should change the mobility.

It is true that the mobility decreases in the series: adenine, 9-methyladenine, 6-N,N-dimethylaminopurine, and adenosine—all in the mono-protonated forms. That 7-methyladenine does not fit into the series can be
<table>
<thead>
<tr>
<th>System</th>
<th>Z</th>
<th>pH</th>
<th>$u \times 10^4$</th>
<th>System</th>
<th>pH</th>
<th>$u \times 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenine; $H^+$</td>
<td>+1</td>
<td>1.60</td>
<td>1.77</td>
<td>adenine; $Hg^{+2}$</td>
<td>1.60</td>
<td>2.14</td>
</tr>
<tr>
<td>7-methyladenine; $H^+$</td>
<td>+1</td>
<td>1.60</td>
<td>1.37</td>
<td>9-methyladenine; $H^+$</td>
<td>2.30</td>
<td>1.45</td>
</tr>
<tr>
<td>9-methyladenine; $H^+$</td>
<td>+1</td>
<td>2.30</td>
<td>1.53</td>
<td>9-methyladenine; $Hg^{+2}$</td>
<td>2.30</td>
<td>1.45</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $H^+$</td>
<td>+1</td>
<td>1.60</td>
<td>1.27</td>
<td>6-N,N-dimethylaminopurine; $Hg^{+2}$</td>
<td>1.60</td>
<td>1.62</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $H^+$</td>
<td>+1</td>
<td>2.30</td>
<td>1.33</td>
<td>6-N,N-dimethylaminopurine; $Hg^{+2}$</td>
<td>2.30</td>
<td>1.40</td>
</tr>
<tr>
<td>cytidine; $H^+$</td>
<td>+1</td>
<td>1.60</td>
<td>0.93</td>
<td>cytidine; $Hg^{+2}$</td>
<td>1.60</td>
<td>1.50</td>
</tr>
<tr>
<td>cytidine; $H^+$</td>
<td>+1</td>
<td>2.30</td>
<td>1.00</td>
<td>cytidine; $Hg^{+2}$</td>
<td>2.30</td>
<td>1.05</td>
</tr>
<tr>
<td>cytidine; $H^+$</td>
<td>+1</td>
<td>2.14</td>
<td>1.12</td>
<td>adenosine; $Hg^{+2}$</td>
<td>2.30</td>
<td>0.74</td>
</tr>
<tr>
<td>deoxyadenylic acid (5');$H^+$</td>
<td>-1</td>
<td>5.00</td>
<td>0.313</td>
<td>adenosine; $Hg^{+2}$</td>
<td>2.30</td>
<td>0.76</td>
</tr>
<tr>
<td>deoxyadenylic acid (5');$H^+$</td>
<td>-2</td>
<td>8.06</td>
<td>1.30</td>
<td>adenosine; $Hg^{+2}$</td>
<td>2.30</td>
<td>0.73</td>
</tr>
</tbody>
</table>

$[\text{adenine } Hg\text{-CH}_3]^{+} \quad u = 1.25 \times 10^{-4}$

$[\text{adenine } (Hg\text{-CH}_3)_2]^{+} \quad u = 0.74 \times 10^{-4}$
rationalized by supposing that the methyl group interferes with the solva-
tion of the amino group.

The fact that positive adenosine has a greater mobility than uni-
negative adenylic acid suggests that the greater charge localization on
the phosphate group in the latter case leads to greater frictional drag.

Cytidine has a lower mobility than adenosine although it is smaller;
this too may be due to charge localization effects.

The factors which affect the mobility are evidently complex. Never-
theless, with the limited information available, we shall attempt to draw
some conclusions about the mercury complexes.

As already indicated in the previous section we believe that at
pH 2.30 the charge on the adenosine-mercury complex is +1, the conclusion
being based on the spectra obtained by PBZVD and on the comparison of
the bonding tendencies of adenine and adenosine. The decrease in mobility
from 1.12 to 0.74 x 10^{-4} in what would be expected for replacing a proton
by a bulky -HgOH group. There is a further decrease on changing to the
CH_{3}Hg^{+}-group.

The increase in mobility of the Hg^{2+}-adenine complex is therefore
unexpected on the basis of the PBZVD conclusion that the complex is
adenine -Hg-OH^{+}. Since the mobilities of (adenine H)^{+} and (adenine-HgCH_{3})^{+}
are 1.77 x 10^{-4} and 1.20 x 10^{-4} we would expect adenine Hg-OH to be about
1.40 x 10^{-2}. The observed mobility of 2.14 x 10^{-4} indicates that indeed
the interpretation of the spectrophotometric data are not always reliable
and at pH 1.60 the complexes (adenine-Hg)^{++} and (adenine-HgOH)^{+} are both
present in significant amounts.
One possible complication should be mentioned here. The electrophoretic data were taken at 6.0° in mixed $H_2O-D_2O$ solvent; the spectrophotometric data were taken at ~27° C in $H_2O$. It is possible that a disagreement between the two kinds of data is due to this factor.

Bearing in mind the discussion in the previous section, we further propose in view of the electrophoretic results that at pH 2.30 the 9-methyladenine complexes are partially in the plus one and partially in the plus two state, but with the greater amount being present as the plus one ion.

The solubility and equilibrium characteristics are such that the 6-N,N-dimethylyaminopurine-mercury system can be studied at pH's of both 1.60 and 2.30. The results in Table 14 can be explained by assuming the following equilibrium constant and mobilities

\[
K = 1.0 \times 10^{-2} \quad + H^+ \quad (20)
\]

\[
u_x = 1.34 \times 10^{-4}
\]

\[
u_y = 1.15 \times 10^{-4}
\]

FBCYVD report the following equilibrium for cytidine,

\[
K = 6.0 \times 10^{-3} \quad + H^+ \quad (21)
\]
At pH 1.60 the ratio of CdHg\(^{++}\)/CdHg-OH\(^{+}\) is calculated to be 4.2:1. At pH 2.30 it is 1:1.2. The corresponding calculated mobilities from Table 1 are 1.75 \times 10^{-4} and 0.54 \times 10^{-4}. These numbers are respectively slightly too high and too low. This indicates that either there is inaccuracy in the data or other reactions besides the assumed one are taking place. Nevertheless, the qualitative feature of the decrease in mobility with increasing pH is satisfactory.

Thus we see that electrophoretic data permit us to make significant qualitative, but not quantitative, statements about the complex species present.
VIII. MISCELLANEOUS RESULTS

In Table 15 are listed a number of mobility measurements not directly related to the discussion of section VII.

TABLE 15

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>T°C</th>
<th>Gradient</th>
<th>pH</th>
<th>( u - \frac{\text{cm/sec}}{\text{volt/cm}} \times 10^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. adenine; ( H^+ )</td>
<td>0.0 ± 0.1</td>
<td>sucrose</td>
<td>2.14</td>
<td>1.09</td>
</tr>
<tr>
<td>2. adenine; ( H^+ )</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>1.60</td>
<td>1.77</td>
</tr>
<tr>
<td>10. adenosine; ( H^+ )</td>
<td>0.0 ± 0.1</td>
<td>sucrose</td>
<td>2.14</td>
<td>0.777</td>
</tr>
<tr>
<td>11. adenosine; ( H^+ )</td>
<td>6.0 ± 1.0</td>
<td>sucrose</td>
<td>2.14</td>
<td>0.905</td>
</tr>
<tr>
<td>12. adenosine; ( H^+ )</td>
<td>8.0 ± 0.3</td>
<td>sucrose</td>
<td>~7</td>
<td>0.00</td>
</tr>
<tr>
<td>14. adenosine; ( H^+ )</td>
<td>10.0 ± 0.5</td>
<td>sucrose</td>
<td>2.14</td>
<td>1.14</td>
</tr>
<tr>
<td>15. adenosine; ( H^+ )</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>2.14</td>
<td>1.12</td>
</tr>
<tr>
<td>22. 7-methyladenine; ( H^+ )</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>1.60</td>
<td>1.67</td>
</tr>
<tr>
<td>23. 7-methyladenine; ( Hg^{+2} )</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>1.60</td>
<td>1.45</td>
</tr>
<tr>
<td>24. 7-methyladenine; ( Hg^{+2} )</td>
<td>7.0 ± 0.1</td>
<td>( D_2O )</td>
<td>1.60</td>
<td>1.8</td>
</tr>
<tr>
<td>25. 7-methyladenine; ( Hg^{+2} )</td>
<td>6.2 ± 0.1</td>
<td>( D_2O )</td>
<td>1.60</td>
<td>2.50</td>
</tr>
<tr>
<td>39. DNA (native)</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>8.0</td>
<td>1.83</td>
</tr>
<tr>
<td>40. DNA (denatured)</td>
<td>6.0 ± 0.1</td>
<td>( D_2O )</td>
<td>8.0</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Experiments 10, 11, and 14 give a rough idea of the increase in mobility as one raises the temperature while keeping all other conditions identical. The velocity for adenosine increased by 50% for a ten degree change. Therefore for the rest of the experiments the temperature was controlled to 0.1° to assure mobilities accurate to 1 or 2 per cent.
As a first approximation on changing the gradient from sucrose to D$_2$O the mobility should be unaffected. The increase observed in experiments 11 and 15 then could be due to errors in the values of the electric fields $E$ that were used.* Another possibility is that the adenosine interacts with the sugar making the molecular volume larger thus reducing the velocity. The same effect is observed in the case of adenine. The increase in mobility is too large in going from the conditions in experiment 1 to those in experiment 2 to be accounted for by the 6° increase in temperature.

The 7-methyladenine-mercuric ion system turned out to be more complex than the others. The mobility changes as a function of time. Experiments 25, 24, and 23 were done immediately after mixing of the solutions, after 10 hours, and after 7 days respectively. In view of the fact that protonated 7-methyladenine has a mobility of $1.87 \times 10^{-4}$ it would appear that the change in mobility for the mercuric complex in going from $2.50 \times 10^{-4}$ to $1.45 \times 10^{-4}$ could be accounted for by a +2 species reacting to form a +1 species. There are also spectral changes but they are much slower and do not seem to be related to the decrease in mobility. Since the hydrolysis of mercuric ion-purine systems is not accompanied by large changes in spectra one is tempted to say that for some unexplainable reason in the case of 7-methyladenine this hydrolysis is slow. The change in spectrum on the other hand could be caused by mercuration in the number 3-position.

The calf thymus DNA experiments were used to check that there is no significant spreading of the boundary due to convection since on the

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*In experiment 11 $E$ was measured directly while in 15 it was calculated from conductivities.
time scale used here these high molecular weight molecules should not diffuse significantly. This was in fact observed. At the same time it was shown that the mobility is the same whether the boundary is moving into or out of the density gradient. Any possible significance of the DNA results is not discussed here since P. Baine and B. M. Olivera of this laboratory are carrying out a much more extensive investigation of the electrophoresis of different DNA's under various conditions.

Table 16 lists the experiments that show the type of reproducibility that can be obtained for the proton and mercuric ion systems. Considering the complexity of the systems involved and also of the solutions the average deviation of approximately 3 per cent is not unexpected.
<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>$u - \frac{cm/sec}{v/cm} \times 10^4$</th>
<th>$\Delta %$ (maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenine; $H^+$</td>
<td>6.0</td>
<td>$2.14$</td>
<td>1.09</td>
</tr>
<tr>
<td>adenine; $H^+$</td>
<td>6.0</td>
<td>$2.14$</td>
<td>1.09</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $H^+$</td>
<td>6.0</td>
<td>$1.60$</td>
<td>1.27</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $H^+$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>1.33</td>
</tr>
<tr>
<td>cytidine; $H^+$</td>
<td>6.0</td>
<td>$1.60$</td>
<td>0.980</td>
</tr>
<tr>
<td>cytidine; $H^+$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>1.00</td>
</tr>
<tr>
<td>adenine; $CH_3Hg^+$</td>
<td>6.0</td>
<td>$4.73$</td>
<td>1.04</td>
</tr>
<tr>
<td>adenine; $CH_3Hg^+$</td>
<td>6.0</td>
<td>$4.73$</td>
<td>1.00</td>
</tr>
<tr>
<td>adenine; $CH_3Hg^+$</td>
<td>6.0</td>
<td>$4.73$</td>
<td>1.05</td>
</tr>
<tr>
<td>adenosine; $Hg^{+2}$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>0.744</td>
</tr>
<tr>
<td>adenosine; $Hg^{+2}$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>0.762</td>
</tr>
<tr>
<td>adenosine; $Hg^{+2}$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>0.727</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $Hg^{+2}$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>1.40</td>
</tr>
<tr>
<td>6-N,N-dimethylaminopurine; $Hg^{+2}$</td>
<td>6.0</td>
<td>$2.30$</td>
<td>1.40</td>
</tr>
</tbody>
</table>
IX. CHARGES AS DETERMINED BY ELECTROPHORESIS AND DIFFUSION

As already mentioned in the introduction the charge \( Z \) of an ion is given by the equation

\[
Z = \left( \frac{kT}{e} \right) \frac{u^o}{D^o} \]

(22)

\[ k = \text{molecular gas constant} \]
\[ T = \text{absolute temperature} \]
\[ e = \text{electronic charge} \]
\[ u^o = \text{mobility} \]
\[ D^o = \text{diffusion coefficient} \]

The above relationship holds strictly only for an isolated particle of charge \( Z \) moving in a dielectric medium under the influence of an electric field \( E \). For charged species in solution the above equation has to be modified because of the ion atmosphere. \( u^o \) and \( D^o \) are measured only in the limiting case of very low concentrations.

The two factors affecting \( u \) are a) the relaxation effect and b) the electrophoretic effect. These are drags experienced by the ion because of retarding forces due to a non-symmetrical ion atmosphere and solvent motion respectively. The Debye-Hückel approximations are used in calculating the magnitude of corrections necessary.

The force \( \Delta F \) due to the dissymmetry of the ion atmosphere is obtained approximately \((8)\) by multiplying the ratio of the distance the ion \( j \) is ahead of its atmosphere to the thickness of the latter by the total force between the ion and its atmosphere,

\[
\Delta F_j = - \frac{F_j}{\kappa T} \frac{F_j}{kT} \]

(23)

\[ \kappa = \text{reciprocal of ion atmosphere} \]
\[ F_j = e_j E \]
The ion of interest will be also carried by the medium in the direction opposite to its motion. If one assumes that the entire charge \(-e\) of the atmosphere is situated on a spherical shell \(1/k\) from the ion and that the motion of the sphere obeys Stokes Law then

\[
\Delta u_j = -\frac{F_j}{6\pi\eta}
\]  

(24)

Consequently the net velocity \(u_j\) is given by

\[
\begin{align*}
\dot{u}_j &= u_j^0 - \frac{\Delta F_j}{kT} + \Delta u_j \\
\text{or} \quad \dot{u}_j &= u_j^0 - \frac{e^2 k F_j}{kT} - \frac{kF_j}{6\pi\eta} \\
\dot{u}_j &= u_j^0 \left( 1 - \frac{e^2 k}{kT} - \frac{KF_j}{6\pi\eta} \right)
\end{align*}
\]  

(25)

The above equation holds at relatively low concentrations so that further refinements are necessary.

The effect of increased ionic strength on diffusion constants cannot be explained by any simple theory. The diffusion constant used in equation 22 is the limiting case for tracer or self-diffusion at low ionic strength. These conditions are only approximately satisfied in the actual experiments that have been done.

The above discussion serves to illustrate the inherent difficulty of calculating \(Z\) for an ion even if accurate measurements of \(u\) and \(D\) are available. However if good accuracy were obtainable then an empirical method might be used. One could calculate an apparent charge \(Z_a\) by use of formula 22 for a compound or complex of definitely known charge. Then if another compound of similar structure had the same \(Z_a\) under approximately
the same conditions one would infer they also had the same actual charge. Such an attempt has been made for the purine systems.

Table 17 gives the diffusion results measured for the stationary boundary and the $Z_a$ as calculated from equation 22. The last column shows the actual $Z$ for the protonated species. The numbers in brackets are the charge ranges expected for the Hg$^{+2}$ complex systems as deduced by electrophoresis. It also should be noted that the stationary boundary diffusion constants were used since the moving boundary ones generally were larger due to some convective or mechanical stirring.

**TABLE 17**

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>$D_a \times 10^6$</th>
<th>$u \times 10^6$</th>
<th>$Z_a$</th>
<th>$Z$(actual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. adenine; $H^+$</td>
<td>2.1</td>
<td>1.09</td>
<td>1.19</td>
<td>+1</td>
</tr>
<tr>
<td>3. adenine; Hg$^{++}$</td>
<td>2.50</td>
<td>2.14</td>
<td>2.03</td>
<td>[1.3-1.7]</td>
</tr>
<tr>
<td>10. adenosine; $H^+$</td>
<td>1.4</td>
<td>0.777</td>
<td>1.50</td>
<td>+1</td>
</tr>
<tr>
<td>17. adenosine; Hg$^{++}$</td>
<td>2.54</td>
<td>0.762</td>
<td>0.72</td>
<td>[1.0-1.2]</td>
</tr>
<tr>
<td>20. deoxyadenylic acid (5'); $H^+$</td>
<td>2.27</td>
<td>0.813</td>
<td>-0.86</td>
<td>-1</td>
</tr>
<tr>
<td>21. deoxyadenylic acid (5'); $H^+$</td>
<td>2.13</td>
<td>1.30</td>
<td>-1.45</td>
<td>-2</td>
</tr>
<tr>
<td>22. 7-methyladenine; $H^+$</td>
<td>3.91</td>
<td>1.87</td>
<td>1.15</td>
<td>+1</td>
</tr>
<tr>
<td>26. 9-methyladenine; $H^+$</td>
<td>3.37</td>
<td>1.58</td>
<td>1.12</td>
<td>+1</td>
</tr>
<tr>
<td>27. 9-methyladenine; Hg$^{+2}$</td>
<td>2.74</td>
<td>1.45</td>
<td>1.27</td>
<td>[1.0-1.4]</td>
</tr>
<tr>
<td>28. 6-N,N-dimethylaminopurine; $H^+$</td>
<td>2.21</td>
<td>1.27</td>
<td>1.33</td>
<td>+1</td>
</tr>
<tr>
<td>29. 6-N,N-dimethylaminopurine; $H^+$</td>
<td>3.03</td>
<td>1.33</td>
<td>1.03</td>
<td>+1</td>
</tr>
<tr>
<td>30. 6-N,N-dimethylaminopurine; Hg$^{+2}$</td>
<td>2.14</td>
<td>1.62</td>
<td>1.82</td>
<td>[1.4-1.8]</td>
</tr>
<tr>
<td>32. 6-N,N-dimethylaminopurine; Hg$^{+2}$</td>
<td>2.06</td>
<td>1.40</td>
<td>1.63</td>
<td>[1.2-1.6]</td>
</tr>
<tr>
<td>35. cytidine; $H^+$</td>
<td>2.28</td>
<td>1.00</td>
<td>1.58</td>
<td>+1</td>
</tr>
</tbody>
</table>
It should be noted that for the protonated species of charge +1 the values of $Z_a$ are in the range of 1.03-1.53. As has been mentioned before the accuracy of the diffusion constants is only of the order of 30% so that the above spread in $Z_a$ is probably due in larger part to the unreliability of the D's. The two negative species shown in Table 17 have low values of $Z_a$ as compared to $Z$. This observed difference between the positive and negative ions probably reflects the different effect that the surrounding medium has on their diffusion constants.

The empirical method as outlined in this section really looks hopeful when one observes that the average $Z_a$'s for the complex mercuric ion systems fall in the expected range as again determined by the electrophoresis results and the $Z_a$'s observed for protonated systems. As an example adenine-Hg$^{+2}$ gives a $Z_a$ of 2.03 while the actual average $Z$ is probably in the range 1.3-1.7. Again we see that for positive ions $Z_a$ tends to be higher than $Z$.

Thus we may conclude that the results obtained so far show that the empirical approach for determining the charge should not be abandoned. Better diffusion coefficient measurements hold the key for determining how good this method may be.
X. GENERAL CONCLUSIONS

The purine and pyrimidine systems involving reaction with Hg$^{+2}$ or CH$_3$-Hg$^+$ have turned out to be more complicated than was suspected from the initial spectrophotometric work. Electrophoresis has suggested that mixtures of complexes exist under conditions where it was believed that only one complex predominates. Further complications such as mercuration and ultraviolet light induced reaction have also been detected. However it appears that even under these somewhat adverse conditions electrophoresis can be a valuable tool in helping to determine what takes place in systems where other types of measurement are hard or impossible.
REFERENCES


APPENDICES
APPENDIX 1

Detailed Description of Apparatus

For convenience, Figure 2, the schematic drawing of the apparatus, is also shown on page 68.

The light source (a) is a General Electric H85 A3 mercury arc having a quartz envelope about 1.5 cm from the arc itself. The arc can be adjusted horizontally and vertically for maximum intensity by means of a rack and pinion arrangement. Directly in front is a 3.5 cm diameter and ~3 mm thick ground quartz plate (b). This light diffuser causes the loss of about 40% of the intensity but averages out all the fluctuations in intensity due to the wandering of the arc. Mounted right next to the quartz plate is a diaphragm type slit (c). The size of the opening can be easily varied and for the experiments to be described here was opened 3 mm. A smaller diameter hole does not allow enough light to pass through while a larger one leads to focusing problems since one does not have any more a point source. As far as the rest of the optical system is concerned the iris of the diaphragm appears to be the light source.

In order to filter out all wave lengths except those in the 240-280 μm range where the compounds of interest have absorption maxima three different filters (d), (e), and (f) are employed. (d) is a 7-54 Corex filter two inches square and 3.04 mm thick (no. 9353). (e) and (f) are identical size (8 cm long; 3.5 cm in diameter), quartz, "high-pressure" filters obtained from the Spinco Division of Beckman Instruments and filled with Cl₂ and Br₂ gas respectively. Thus most of the light intensity that has been utilized in these experiments is due to the 265 μm line with
Fig 2

Electrophoresis Apparatus
(Schematic)

d - mercury arc
b - quartz light diffuser
c - slit
d, e, f - filters
g, h, i, j - quartz lenses

h, k - quartz windows
i - cooling cell
j - electrophoresis cell
m - moving slit
o - phototube

1.8 cm | 9 cm | 15 cm | 8 cm | 8 cm
30.3 cm

14.8 cm | 52.7 cm | 60 cm
30 cm

15.5 cm

potentiometer

stirrer

defogging

insulation

defogging

to battery
and x-y recorder

to power
supply and
x-y recorder
much smaller contributions from nearby emission lines. An actual experimental check was made to show that the light is monochromatic enough so that deviations from Beer's Law are not detectable.

(g) is a 40 mm diameter fused quartz lens with a 28.5 cm focal length for the 265 μm line. Since the apparent light source (c) is at the focus parallel light leaving the lens enters the cooling cell at point (h) through a quartz window having a diameter of 5 cm.

The cooling cell (i) is made from ~1.5 mm thick stainless steel plate. It is 30 cm long, 25.5 cm wide, and 30 cm deep and can be lowered and raised as necessary. Mounted flush with the inside walls is the cooling coil consisting of 24 feet of 5/16" o.d. stainless steel tubing which is connected to a Precision Scientific Company constant temperature bath. By this means the temperature of the distilled H₂O in the cooling cell can be maintained to within ± 0.1° C of any preset temperature. The cooling cell is provided with a variable speed stirrer and is completely insulated by 1" thick polystyrene foam. At position (k) is another quartz window 5 cm in diameter. For experiments below room temperature predried air is passed continuously over the windows to prevent fogging.

The electrophoresis cell which will be described separately is mounted at (j) 14.8 cm from window (h). The parallel light obtained at (g) passes through the 1 cm light path of the electrophoresis cell and leaves the cooling cell by window (k). 52.7 cm from position (j) is a quartz lens (l) similar to lens (g). The 1:1 image of the electrophoresis cell is formed 60 cm from (l) at (m) where the moving slit is located. The object and image distances are not equal because of the water in the light path.
The moving slit is raised and lowered manually by means of a copper strip which winds around the axle of a linear, precision, single-turn, continuous-rotation 10 K potentiometer (G. S. Marshall Company—Helipot Model No. 5705). A 1.5 v battery is used as a voltage source. The almost linear change in current as the slit is moved is plotted as the abscissa on a Moseley Model X-135 x-y recorder. Absolute distance is obtained from markers which are a definite distance apart next to the electrophoresis cell. The slit width is adjustable but has been set at 0.1 mm.

At position (n) 1.5 cm past the slit is a 15.5 cm focal length lens. It focuses the light passing through the slit on an RCA IP28 phototube. The lens is used in order to try to keep the light falling on the same spot of the photosensitive surface no matter where the moving slit is positioned.

In the photomultiplier there are ten stages. The stage resistances are 68 K, there is a load resistor of 225 K and in parallel with the load resistor there is a .05 μfd capacitor. A Keithley Model 240 high voltage supply has been used to give a 60 volt drop per stage. The final photocurrent is proportional to the amount of light reaching the photocell and is measured by using the y-input of the x-y recorder. The input resistance of the x-y recorder is in parallel with the photocell load resistor.

Thus one obtains a plot of intensity of light passing through the electrophoresis cell versus position in the cell.

By choosing the correct light filters and phototubes and with proper adjustment for the change in focal length as a function of wavelength the apparatus could be used for investigation of systems having absorption maxima in other regions of the ultraviolet and in the visible.
APPENDIX 2

Detailed Description of the Filling of the Electrophoresis Cell

For convenience Figures 3 and 4 and Table 2 on all of which the following discussion is based are also included in this appendix.

1. The electrophoresis cell is mounted and clamped in the proper position in the cooling cell. The cell should be clean and dry.

2. Electrode compartment K is filled through stopcock I with electrolyte solution (no. 8) care being taken that no air bubbles remain trapped in K or stopcock J. Stopcock J is closed right after solution 8 pushes all air out of it, but I remains open. Syringes are used for layering all the solutions in the cell.

3. Polyethylene tubing L leading from the mixer to the inlet tube E is pinched closed.

4. Solution No. 1 is passed in through stopcock H until it is just past joint C. Step 3 prevents solution 1 from creeping into the inlet tube.

5. Stopcocks H and I are closed and J is opened. Distilled H₂O is passed into the cooling cell and is cooled down to the desired temperature. Simultaneously defogging of windows is started.

6. Solution 1 is added or removed as necessary so that its surface is even with the inlet tube.

7. The syringes containing solutions 2 and 4 are mounted in the gradient making machine.

8. Inlet tube L is opened. Tubing M is filled with solution 2 and tubing N, the mixer, tubing L, and the inlet tube E are filled with
Electrophoresis Cell

- Ag-AgCl electrode
- 18-28% D$_2$O gradient with absorbing material (no. 5)
- 36% D$_2$O solution (no. 1)
- 36% D$_2$O solution passed in here
- 8-28% D$_2$O gradient passed through here (no. 2-5)
- density gradient machine
- mixer
- N
- L
- Al
- B
- C
- optical path (quartz)
- wire markers
- 18-28% D$_2$O gradient with absorbing material (no. 2-5)
- 4% D$_2$O solution (no. 6)
- 8-18% D$_2$O gradient (no. 2, 4)
- 0% D$_2$O solution (no. 7)
- level of cooling water
Fig 4

7-Methyladenine; H^+
← + ions

(I_b) - base line is parallel to the x-axis at 0 intensity units

Intensity (I) with respect to base line (I_b)
TABLE 2

A Representative Set of Solutions for an Electrophoresis Experiment

<table>
<thead>
<tr>
<th>Soln. no.</th>
<th>D$_2$O vol. %</th>
<th>NaClO$_4$ F</th>
<th>HClO$_4$ F x 10$^3$</th>
<th>Hg(ClO$_4$)$_2$ F x 10$^4$</th>
<th>Purine or Pyrimidine F x 10$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
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</tr>
<tr>
<td>2</td>
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<td>-</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.10</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
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<td>8.0</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
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<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.10</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

8. Electrolyte: ~ 6 F NaCl and 0.6 F HCl

solution 4. L is pinched closed so that there is no tendency for solution 4 to enter the cell.

9. Now using a syringe that has attached to the needle tip a narrow glass capillary bent at right angles one carefully layers solution 6 on top of solution 1 until the cell is filled to the top of joint B. This solution has been precooled to the temperature in the cell so that no convection currents are set up.

10. The top electrode is mounted in place and electrolyte solution is placed in compartment A until the electrode is just covered.

11. Precooled solution 7 is carefully layered on top of solution 6 to the top of the square central tube and similarly on top of the electrolyte in compartment A. Then additional volume is added as shown to obtain good electrical contact.
12. Now the initial intensity \( I_0 \) is measured (see Fig. 4). The mercury arc is allowed to warm up at least a half-hour before the filling of the cell.

13. Polyethylene tube L is opened and the density gradient machine is turned on. At the halfway mark the machine is stopped, L is closed again and the syringes with solutions 2 and 4 are replaced by syringes containing solutions 3 and 5. Care has to be taken that no bubbles are introduced into the system during this operation.

14. Tube L is opened and the rest of the gradient passed in until the boundary appears in the light path (curve 1 - Fig. 4). Now L is clamped shut once more. Since about two-thirds of L are submerged in the coolant no precooling of solutions 2 to 5 is necessary.

15. If diffusion measurements are desired then the boundary is sharpened by withdrawal of solution between points x and y (Fig. 4). A very narrow capillary whose end is bent at 90° and which is attached to a syringe clamped in a precalibrated position is used. After the withdrawal of the solution the capillary can be removed with minimum disturbance giving a boundary which can be as narrow as 0.2-0.3 mm (curve 2 - Fig. 4).

16. One is now ready for electrophoresis.
PROPOSITIONS
PROPOSITION I

It is proposed that an investigation be made of the interaction of deoxyribonucleic acid (DNA) with methylmercury.

The metal ion complexes of DNA have drawn increasing attention during the past few years as a possible means of obtaining additional information about the structure and properties of DNA. Hg$^{+2}$ and Ag$^{+1}$ ions have been used to study the complexing of the nitrogen base moieties (1-6). Various types of measurements have been made, but so far it has not been possible to propose a definite complex or complexes to explain all the observed results.

As Hg$^{+2}$ ion is added to DNA at pH 5.7 a decrease in viscosity is observed indicating some disruption of the helical DNA structure. The complexing is accompanied by spectral shifts. Initially there seem to be two protons released per Hg$^{+2}$ ion. There is one complex up to one Hg$^{+2}$ ion per two DNA bases and additional complex or complexes at higher concentrations. The complexing can be reversed by the addition of a strong Hg$^{+2}$ complexing agent and native DNA with retained biological activity is recovered (7).

The hydrogen bonding of an A-T pair in DNA is shown below as a supplement to the discussion that follows.
Reaction of Hg$^{+2}$ with the N-3 or N-7 nitrogens of adenosine (or guanosine in the GC pairs) cannot give the observed two proton release. An Hg$^{+2}$ ion between the bases of the Watson-Crick structure should destroy all the hydrogen bonding. A bond between N-3 of the pyrimidine and N-1 of the purine should liberate only one proton, while a bond between N-3 of the pyrimidine and the amino group of the purine would give a two proton release but requires a large amount of distortion of the DNA.

$x$-interaction of the Hg$^{+2}$ ion with two bases vertically above each other in the same strand would also lead to distortion of the DNA and leaves unanswered the question of the observed proton release.

Similarly, the suggestion that the two strands of DNA slide so that thymidines can be opposite each other would give the initial two proton release but requires a large amount of hydrogen bond cleavage.

Finally, the fact that native DNA can be regenerated would seem to rule out any suggestion that any of the complexes are the result of strand separation.

The interaction of Ag$^{+}$ ion with DNA is quite different from that of Hg$^{+2}$. There is very little decrease in viscosity, indicating that the helical structure is not disrupted. There is no proton release upon formation of the first complex ($\sim \frac{1}{5}$ bases) and some proton release for the second complex ($\frac{1}{2}$ bases). As pointed out by Yamane and Davidson (4), the above can be explained by assuming reaction with N-7 or N-3 nitrogens of the purines in alternate base pairs, followed by further reaction with the remaining N-7 or N-3 accompanied by some replacement of the hydrogen bonding thus liberating some H$^{+}$ ions.

It is obvious that the interactions of the Hg$^{+2}$ and Ag$^{+}$ ions with DNA are complex. By simplifying the system, i.e., by using an ion like
CH₃-Hg⁺ which has only one reactive position it should be possible to obtain more concrete evidence about the type of complex or complexes being formed. Also the problem of hydrolysis present in the Hg⁺²-DNA system is eliminated.

For the methylmercury-DNA system it should be easy to tell whether the reaction takes place at the N-3 or N-7 nitrogens of the purines as opposed to interaction with the nitrogens involved in the hydrogen bonding. In the latter case one should obtain strand separation.

Thus it is felt that experiments of the type already carried out for the Hg⁺²-DNA and Ag⁺-DNA system should be extended to the CH₃Hg⁺-DNA system.

REFERENCES TO PROPOSITION I

4. T. Yamane and N. Davidson, Biochimica et Biophysica Acta (1962), 55, 609-621.
PROPOSITION II

It has been observed in this laboratory that adenosine in the presence of excess \(\text{Hg}^{+2}\) ion undergoes photochemistry. It is proposed that the type of reaction taking place and the type of products formed be investigated.

Since photochemistry may play an important role in many biological processes the effect of radiation on biological materials and their components has been of interest. A limited amount of experiments have been done with purine derivatives.

Purine nucleoside-bound carbohydrates like adenosine are stable even in alkalai where mono- and polysaccharides decompose. Furthermore, Shugar and Wierzchowski (1) showed that for adenosine in 0.01 M NaOH the sugar moiety is essentially unaffected by irradiating a \(10^{-4}\) \(\text{F}\) solution with a resonance lamp (all wavelengths below 240 \(\mu\) filtered) at an intensity of \(10^{17}\) quanta/cm\(^2\)/min for 1 hour. Thus one concludes that the carbohydrate components of purines are unaffected by radiation of wavelengths greater than 240 \(\mu\) and even those down to 220 \(\mu\) where the carbohydrates start absorbing weakly. The destruction of the sugar may occur in some cases by energy transfer from the aromatic ring. Other investigators (2-4) have also shown that nucleosides and nucleotides of adenine and guanine are remarkably resistant to irradiation but that under analogous conditions adenosine triphosphate undergoes considerable degradation. However no adenosine but only adenine is among the photoproducts indicating that the sugar adenine bond is made labile by the pyrophosphate group.
Since adenosine in general exhibits high resistance to photo-decomposition it would be of interest to determine what sort of products are obtained when the Hg$^{+2}$ complex undergoes photoreaction.

There are four main possibilities of which I prefer the initial one.

a) That the Hg$^{+2}$ ion makes the sugar-purine bond labile. Then the products should be an adenine-Hg$^{+2}$ complex and carbohydrate. There is also the possibility that the liberated carbohydrate reacts with the excess Hg$^{+2}$ ion.

b) That the carbohydrate group is destroyed and the adenine-Hg$^{+2}$ complex is left intact.

c) That the purine is destroyed leaving the carbohydrate to possibly react further with the excess Hg$^{+2}$ ion.

do) That there is addition of H$_2$O across a double bond as in the case of pyrimidines (as first observed by Sinsheimer with uridylic acid (5)) or that some related reaction takes place.

Besides determining the products it would be of interest to see whether compounds similar in structure to adenosine are also made photosensitive when complexed with Hg$^{+2}$ ion.

REFERENCES TO PROPOSITION II


PROPOSITION III

The determination of the amount of phosphorous (P) in different materials of biological interest is often involved, tedious and unreliable (1).

It is proposed that the amount of $^{31}$P in these materials be determined by the use of neutron activation analysis.

Irradiation of samples containing $^{31}$P in a thermal neutron pile leads to the production of radioactive $^{32}$P. This isotope has a half-life ($t_1$) of 14.30 days and undergoes beta decay (1.701 kev $\beta^-$).

The thermal neutron cross section of $^{31}$P is 0.23 barns. For a neutron flux of $5 \times 10^{12}$ $\text{cm}^{-2} \text{sec}^{-1}$ and an irradiation time of 1 hour the ratio of atoms $^{32}$P/atoms $^{31}$P $\approx 4.14 \times 10^{-9}$.

For $2 \times 10^3$ disintegrations of $^{32}$P/min right after irradiation the number of $^{32}$P atoms needed are

$$ n_{^{32}P} = \frac{(- \Delta P^{^{32}P}/\Delta t)(t_1)}{\ln 2} = 5.95 \times 10^7 \text{ atoms} $$

Then the approximate initial moles of $^{31}$P needed are

$$ n_{^{31}P} = \frac{5.95 \times 10^7 \text{ atoms}}{(4.14 \times 10^{-9})(6.023 \times 10^{23} \text{ atoms/mole})} $$

$$ n_{^{31}P} = 2.38 \times 10^{-8} \text{ moles} $$

This corresponds to $7.6 \times 10^{-8}$ mg $^{31}$P. A deoxyribonucleic acid (DNA) solution having an optical absorbance of 1 at $\lambda = 260$ nm in a 1 cm cell has a P concentration of $\sim 1.5 \times 10^{-4}$ moles/liter or $5 \times 10^{-3}$ mg of P/ml. Therefore under the conditions outlined above the practical limit
would be the analysis of 1 mL of solution having an optical absorbance of 0.1.

The sensitivity of the method can be increased in several ways:

a) Thermal neutron fluxes as high as $1 \times 10^{14}$ are available increasing the sensitivity by a factor of 20.

b) Longer irradiation times can be used. Irradiation times longer than $t_{\frac{1}{2}}$ are not practical because of saturation. However one can easily increase $\frac{dN_{32}}{dt}$ by at least a factor of one hundred.

c) Another factor of ten may be obtained by settling for smaller initial decay rates and correspondingly longer counting times in those cases where the solution is free of any interfering decay of other nuclides.

As a result the detection of 0.01 µg of $^{32}$P is possible. Therefore neutron activation may also be used for the detection of the presence of small amounts of DNA or other materials. Up to now such detection involves the complicated process of labeling the material with $^{32}$P by chemical means.

For calibration purposes one would simultaneously irradiate a solution of known inorganic $^{31}$P concentration. In general a few days wait may be necessary to allow any shortlived radioactivity in the samples to die out ($^{24}$Na for example).

That the neutron activation procedure is feasible is supported by the fact that Mautner et al. (2) have succeeded in labeling the adenosine phosphates with $^{32}$P by irradiation in a thermal neutron flux.

The great advantage of neutron activation analysis is the minimum of chemical manipulation involved and the increased sensitivity that is obtainable.
REFERENCES TO PROPOSITION III


PROPOSITION IV

Ferreira et al. (1) observed that $\text{Hg}^{+2}$ ion in the presence of excess protonated purine or pyrimidine derivatives ($\text{HP}^{+n}$) undergoes the following reaction

$$\text{Hg}^{+2} + 2\text{HP}^{+n} \rightleftharpoons \text{HgP}_2^{+2n} + 2\text{H}^+$$

In view of the mobility experiments already carried out for different $\text{HP}^{+n}$'s in excess mercuric ion (2) it would be of interest to see how the mobilities of the $\text{HgP}_2^{+2n}$ complexes vary as a function of the different purine and pyrimidine derivatives that are available. Therefore it is proposed that the mobilities of the above complexes ($\text{HgP}_2^{+2n}$) be measured using radioactive $\text{Hg}^{203}$ isotope for detection of the concentration profile of the material of interest.

The optical methods used for the detection of concentration profiles cannot be used in this case because the purine and pyrimidine derivatives which have to be present in excess have large absorbances. However if one were to use $\text{Hg}^{+2}$ ions having radioactive $\text{Hg}^{203}$ present as a tracer then the electrophoresis boundary can be detected by careful removal of small aliquots of the solution in the cell followed either by $\beta$ or $\gamma$ counting (203 kev $\beta^-$; 279 kev $\gamma$). Good accuracy should be obtainable if the distance of travel is long enough. Large enough counting rates are easily obtained and the half-life is long enough (45.3 days) so that corrections are not necessary for the decrease in decay rate during electrophoresis.

The determination of the amount of $\text{HgP}_2^{+2n}$ present as a function of position should be easier by the proposed means than by the use of an
analytical method. If the concentration of $\text{Hg}^{203^{++}}$ is high enough the boundary may be roughly detected during electrophoresis.

REFERENCES TO PROPOSITION IV


PROPOSITION V

So far it has not been possible to present any convincing evidence as to the site of binding of a Hg$^{2+}$ ion in a purine-like adenosine where N-1, N-3, N-7 and even N-6 are all possible binding sites.

It is proposed that in the case of adenosine and for other purines and pyrimidine derivatives where the solubility of the Hg$^{2+}$ complex is high enough nuclear magnetic resonance (nmr) measurements should be used as a possible way of obtaining additional information or even solving this problem. Also for easier interpretation of the nmr spectra labeling with $^{13}$C is suggested.

Measurements of proton nmr have been carried out for a number of different purines and pyrimidines in D$_2$O (1) and in deuterated dimethyl sulfoxide (2).

The remainder of the discussion will deal essentially with adenine and adenosine as representative systems.

In D$_2$O the N-6 and N-9 protons of adenine and adenosine exchange too rapidly to be observable. The chemical shifts for the C-2 and C-8 protons are similar because the environment is similar. The chemical shifts obtained by the Jardetzky's (1) are listed in parts per million with respect to the methyl protons of toluene.
solvent  H-2    H-3
adenine  +1.41  -1.62  -1.43
adenosine +1.37  -1.53  -1.73

The assignment of the shifts to the protons in the 2 and 8 positions are not really definite since they were made according to the assumption that the addition of sugar in the 9-position would affect H-8 more than H-2.

Also since in going from neutral adenosine at pH 12.5 to the protonated species at pH 1.82 the chemical shift is larger for H-2.

<table>
<thead>
<tr>
<th>pH</th>
<th>1.82</th>
<th>12.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_8</td>
<td>-2.09</td>
<td>-1.75</td>
</tr>
<tr>
<td>H_2</td>
<td>-2.01</td>
<td>-1.57</td>
</tr>
<tr>
<td>solvent</td>
<td>+1.57</td>
<td>+1.54</td>
</tr>
</tbody>
</table>

protonation next to this position is indicated. It is probably the one position and not the three since protonation takes place in the former in solid adenine hydrochloride (3,4).

In deuterated dimethylsulfoxide the N-6 and N-9 proton resonances are also observed. In the case of Hg^{42} complexes however this solvent is probably not practical because of low solubility and side reactions. At the same time the reactions taking place in an aprotic solvent might not be the same ones that take place in H_2O or D_2O.

First of all in order to confirm the assignment of the peaks in D_2O by the Jardetzky's C^{13} with I = 1/2 should be incorporated in the imidazole ring (i.e., position 8). H-8 would then appear as a doublet while H-2 would still be a singlet. Synthesis of the labeled compound is practical.
Now the addition of $\text{Hg}^{+2}$ ion just like protonation should lead to observable chemical shifts. Larger shift for the doublet would indicate reaction on the imidazole ring while in the reverse case the $\text{Hg}^{+2}$ should be on the pyrimidine part of the molecule.

Determination of the exact position may be possible by comparing the shifts obtained with a number of structurally different purines. The limitation in this approach is probably solubility. It might be difficult to get concentrated enough solutions to detect a signal.

REFERENCES TO PROPOSITION V

PROPOSITION VI

It has been reported recently (1) that crystals of cuprous chloride (CuCl₂) undergo striking changes in color upon heating. At room temperature the crystals are colorless. They change to deep blue at $\sim 130^\circ C$ and to blue-black as the temperature is increased further. The color changes are reversible since cooling leads to the original colorless CuCl₂.

It is proposed that the change in absorption is due to an electron transfer process. As the temperature is raised some of the Cu⁺ atoms may lose an electron to the neighboring Cu⁺ atoms thus creating positive (Cu⁺⁺) and negative (Cu⁰) sites in the crystal. It seems probable that the electrons would be lost preferentially from the Cu⁺ atoms and not the Cl⁻ atoms though the latter possibility should be kept in mind. Then by electron transfer between Cu⁺⁻Cu⁺⁺ and Cu⁰⁻Cu⁺ the positive and negative sites may migrate in the crystal. These processes would account for the observed high absorption in the visible region.

It should be noted that highly absorbing electron transfer complexes of the type (Cl⁻⁻Cu⁺⁻Cl⁻⁻Cu⁺⁺⁻Cl⁻)⁰ have been shown to exist in aqueous solution (2). Therefore it is conceivable that similar processes would take place in the CuCl₂ crystals.

Three types of experiments are suggested to test the proposal:

a) Electron spin resonance.

Cu⁺ and Cl⁻ have all their electrons paired. On the other hand Cu⁺⁺ and Cu⁰ both have an unpaired electron. Thus it should be possible
to see the characteristic Cu$^{+}+$ ($d^9$) e.s.r. signal and another signal due to Cu$^0$ ($s^1$). If the frequency of electron transfer is not too high one may also be able to observe the hyperfine splittings (quartets) resulting from the interaction of the electrons with the nuclear spins ($I = 3/2$). This splitting may be quite substantial for Cu$^0$ since the odd electron spends an appreciable time near the nucleus ($s^1$).

b) Electrical conductivity

If as one increases the temperature electron transfer does take place then the presence and migration of the positive and negative sites should increase the electrical conductivity of the crystal. This effect then may be measured.

c) Doping of the CuCl$_2$ crystals.

One should look at the color changes when one dopes a CuCl$_2$ crystal with Cu$^{+}+$ and heats the result. One should also see whether elemental chlorine is given off upon melting i.e. to see whether Cl$^-$ is possibly involved in the electron transfer.

All of the above experiments should help to resolve the problem of the striking color changes shown by CuCl$_2$ crystals.

REFERENCES TO PROPOSITION VI