PHYSICAL AND INORGANIC CHEMISTRY

On the Complexing of Desoxyribonucleic Acid (DNA) by Mercuric Ion[1]

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The complexing of DNA by mercury(II) is studied. In agreement with the prior investigations by Katz and Thomas, there is a decrease in the intrinsic viscosity and a spectral shift when Hg^{II} adds to DNA. The reaction can be reversed by adding complexing agents for Hg^{II} and the original native DNA recovered. The results indicate that Hg^{++} rather than $HgCl_2$ is being bound and that it is adding to the base moieties, not to the phosphate groups. As Hg^{++} is added, one type of complex with a characteristic spectrum forms up to a ratio of one Hg^{++} to two bases for the several natural DNA's studied (calf thymus, E. coli and M. lysodeikticus), irrespective of the GC:AT ratio in the DNA. With excess Hg^{++}, a second higher complex forms. Protons are released when Hg^{++} adds to DNA at pH 5.7. The initial values of $\Delta H^+/\Delta Hg^{++}$ are in the range of 1.8–2.0. The strength of binding of Hg^{++} as estimated by chloride titrations decreases in the order: AT polymer, heat-denatured calf thymus, native calf thymus, E. coli and M. lysodeikticus. Adding Hg^{++} ion causes a rather small decrease in the denaturation temperature of calf-thymus DNA. The spectral and titration properties of AT polymer are quite different from those of the native DNA's. We are unable to propose a structure for the DNA-Hg^{II} complex which explains all of the above-mentioned properties. It is probable, however, that a considerable degree of order in the base packing is retained in the complex.

Introduction

This work was motivated by the thought that useful and interesting information as to the structure and properties of the nucleic acids can be obtained by a study of their metal ion complexes. At low ionic strengths, the nucleic acids and synthetic polynucleotides form complexes with a number of cations. including Mg^{++},[2,3] Mn^{++}[3] and Cu^{++}.[4] (The references cited are representative but not exhaustive.)

The absence of significant spectral shifts upon complex formation with Mg^{++}, Ca^{++} or Mn^{++}, our general knowledge of complex ion binding, and the studies by Shack and Bynum[2] all indicate that these cations are bound to the negative phosphate groups by electrostatic forces.[5]

There is a general parallelism between the base strength of a series of nitrogen bases and their

affinity for any given transition metal cation. The purines and pyrimidines present in nucleic acids are not very strong bases and would not be expected to bind metal ions strongly. Because of our wish to study the interaction of a metal ion with the base moieties, we have chosen that metal ion which forms the strongest bonds with nitrogen bases, namely, Hg^{++}.[6]

Our work has been limited to the study of the interaction of DNA with Hg^{++}. Discussion of the previous contributions by Katz,[7] Thomas[8] and Pour-El and Dekker[9] will be incorporated into the presentation of results.

Results

Viscometry.—The reaction of calf-thymus DNA with $HgCl_2$ was first reported by Katz,[7] who found a drastic decrease in the viscosity of dilute DNA solutions upon addition of approximately equivalent quantities of $HgCl_2$, even at a total ionic strength of 0.6 M (sodium citrate). This

(1) Taken in part from the Ph.D. Thesis by T. Y., California Institute of Technology, 1960.

(2) J. Shack and B. Bynum. Nature. **184**, 635 (1959).

(3) G. Felsenfeld and S. Huang, Biochim. Biophys. Acta. **34**, 234 (1959).

(4) G. Zubay and P. Doty. ibid., **29**, 47 (1958).

(5) Studies in this Laboratory by P. Brooks indicate that Co^{++} ion is bound by DNA by interaction with the negative phosphate groups.

(6) The studies by Harkins and Freiser (J. Am. Chem. Soc., **80**, 1132 (1958)) show that Cu^{++} binds the anion of adenine but does not bind adenosine.

(7) S. Katz, ibid.. **73**, 2238 (1952).

(8) C. A. Thomas, ibid., **76**, 6052 (1954).
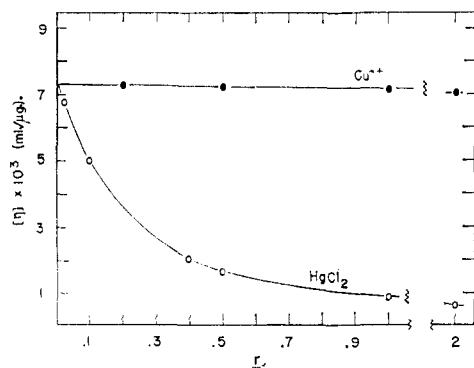
(9) A. Pour-El and C. A. Dekker. private communication.

Fig. 1.—Effect of HgCl₂ on the intrinsic viscosity of calf thymus DNA solution in 0.1 M NaClO₄ and 0.01 F HAc, NaAc, pH 5.7, $T = 25.0°$; r = moles of added HgCl₂ per mole of P. The effect of Cu(ClO₄)₂ under identical conditions is shown for comparison.[4] The effect of HgCl₂ is the same in 0.4 M NaClO₄ solution. An intrinsic viscosity of 7×10^{-3} ml. μg.$^{-1}$ is 70 dl. g.$^{-1}$.

effect is not observed with any other metal ion. Upon addition of excess chloride or cyanide ions, which strongly complex Hg^{II}, the solution regains its initial viscosity. Our own measurements of this phenomenon, which are in complete accordance with Katz's findings, are displayed in Fig. 1. The DNA concentration was 1.5×10^{-4} F in phosphorus or in bases. (F means gram formula weights per liter.) A concentration range of $r = 0$ (r = moles added HgCl₂/moles of P) to $r = 2$ in HgCl₂ was covered. There is a relatively large initial drop in viscosity with r which begins to level off at ca. $r = 1$. Dilution with 0.1 M NaCl completely reverses the effect of the added HgCl₂, in agreement with the observations by Katz.

The effect of Cu(ClO₄)₂ is shown for comparison in Fig. 1. In this case, in order to produce a four-fold decrease in viscosity, an r of the order of 50 is required.[4] The effect of Mg^{++} is much smaller and the intrinsic viscosity of DNA is the same in 0.2 M MgCl₂ as in 0.2 M NaCl.[4]

Transformation Experiment.—Both the viscosity studies and the spectrophotometric experiments reported in the next section show that, by these physicochemical criteria, native undenatured DNA can be recovered from the Hg^{++} complex by addition of reagents which complex the Hg^{++} more strongly. Furthermore, experiments which are reported in detail elsewhere[10] show that the addition of Hg^{++} and its subsequent removal does not affect the biological activity of pneumococcal transforming DNA. Thus, in all respects, the native DNA molecule seems to be recovered unchanged after addition and removal of Hg^{++}.

Spectrophotometry.—The spectrum of calf-thymus DNA in the presence of added HgCl₂ is shown in Fig. 2a. Upon addition of HgCl₂ for $0 < r \leqslant 0.5$, there is a depression of the maximum at 258 mμ, an isosbestic point at 262.5 mμ, and the development of a new maximum at about 268 mμ. On increasing the HgCl₂ concentration above $r = 0.6$, the wave length $\lambda = 262.5$ mμ ceases to be an isosbestic point; there is a new isosbestic point at

(10) W. Dove and T. Yamane, *Biochem. and Biophys. Research Communications*, **3**, 608 (1960).

about 273 mμ, and a maximum at about 275 mμ. As we shall see in detail later, addition of NaCl solution completely reverses these changes and gives rise to the original absorption spectrum of the DNA.

The first and most obvious point is that there are evidently two complexes, one for $0 < r \leqslant 0.5$, that is up to one Hg^{II} per base pair, and the second for $r > 0.5$. The sharpness of the transition at $r = 0.5$ shows that formation of the first species is essentially complete before formation of the second complex begins and that the concentration of free Hg^{++} or HgCl₂ at $r = 0.5$ must be quite low.

The observations (and conclusions) reported above are identical with those first made by Thomas.[8] Thomas also observed an isosbestic point for all r at 238.5 mμ, but this was not the case for our calf-thymus sample. (There is such an isosbestic point at 240 mμ for our *E. coli* DNA sample for $r \leqslant 0.5$, however.) We do not know the reason for the rather minor differences between our results and those of Thomas around 238 mμ and can only suggest that they are due to subtle differences between the preparations used.

Pour-El and Dekker[9] have also confirmed Thomas' spectral observations. Furthermore, they report that the formation of the DNA·Hg^{II} complex and its dissociation by NaCl solution to reform native DNA can be observed by rotatory dispersion measurements. They find no spectral changes and no changes in the rotation when HgCl₂ solution is mixed with poly-deoxyribophosphate, which supports the theory that the mercuric ion is interacting with the purine and/or pyrimidine bases.

Identical spectral shifts and identical viscosity changes are produced if Hg(ClO₄)₂ solutions are used rather than HgCl₂. Thus the reactions observed are due to Hg^{++} ions rather than HgCl₂ molecules. Quantitative experiments bearing on this question are reported in a later section.

As a matter of practical convenience, many of the experiments were done by adding HgCl₂ stock solutions rather than Hg(ClO₄)₂ solutions, because the latter must contain added free acid to prevent hydrolysis to Hg(OH)₂.

Spectra were run with unbuffered solutions or with solutions buffered with 0.01 F acetate buffer. No significant differences in behavior were observed, and the results are similar to those of Thomas in 0.4 F Ac$^-$ buffer and to those of Katz in 0.1 F citrate buffer. However, we have observed spectral shifts of the HgCl₂ spectrum (no DNA present) upon addition of 0.4 F NaAc although not with 0.01 F NaAc. There are undoubtedly weak Hg^{II}-acetate and Hg^{II}-citrate complexes; so that high concentrations of the buffer ions should be avoided for quantitative equilibrium experiments.

Fig. 2b shows the spectrum of a heat denatured, low viscosity sample of calf thymus DNA (90° for 1 hr.). It shows the typical hyperchromic effect with respect to the "native" material. On adding HgCl₂, the same shifts relative to the unmercurated sample are observed as for the native DNA. Upon addition of excess chloride ion, the spectrum of the denatured unmercurated DNA returns. Thus the mercury complex of denatured
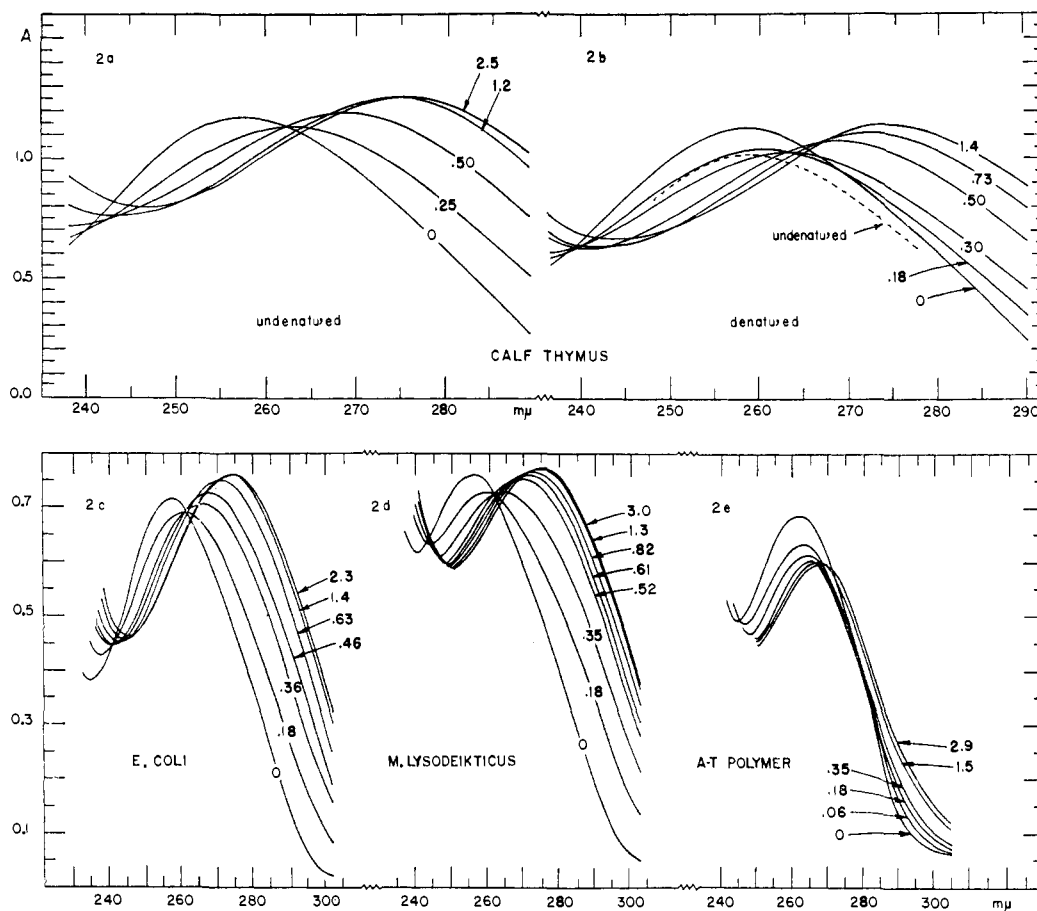
Fig. 2.—Effect of $HgCl_2$ on the ultraviolet absorption spectra of different DNA's: (2a) Calf thymus (%GC = 42); (2b) heat denatured calf thymus DNA ($[\eta]$ = 1.2 × 10^{-3} ml./µg.); (2c) E. coli (%GC = 50); (2d) M. lysodeikticus (%GC = 72); (2e); A-T polymer (%GC = 0). Spectra taken in 0.10 M $NaClO_4$, pH 5.6–5.7 (unbuffered) with increasing r; r = moles of $HgCl_2$/moles of P; except that 2a and 2b were taken in 0.01 F sodium acetate buffer, pH 5.7, 0.10 M $NaClO_4$. The actual concentrations of DNA may be computed from the molar absorptivity (ϵ_-) values in Table II.

DNA shows a similar spectrum but a hyperchromic effect with respect to the mercury complex of native DNA; upon addition of excess chloride, the former reverts to denatured DNA and the latter to native DNA.

The spectra of E. coli DNA (%GC = 50) and M. lysodeikticus DNA (% GC = 72) are displayed in Fig. 2c and Fig. 2d. (Calf-thymus DNA has % GC = 43.) All of the samples show an isosbestic point of about 262 mµ up to r = 0.5 and the formation of a new spectral type of a complex for r > 0.5. Thus these experiments indicate that in the complex with r ≤ 0.5, there is one Hg^{++} per base pair or one Hg^{++} per purine or one Hg^{++} per pyrimidine and eliminate possibilities such as one Hg^{++} per guanosine residue plus one Hg^{++} per cytidine residue, with no Hg^{++} ions attached to adenosine or thymidine residues or the reverse hypothesis.

The spectral changes on adding Hg^{++} to A-T polymer (Fig. 2e) are quite different. There is no isosbestic point, and the shift of the spectrum to longer wave lengths is much less marked. The spectra were reversed by the addition of Cl^-.

We are unable to explain the difference between the natural DNA's and A-T polymer. The A-T polymer had been stored in our cold room in 0.1 M $NaClO_4$ for several months; however, an aliquot taken at the time of use showed a denaturation temperature profile (as observed by spectrophotometry) very similar to that reported by Marmur and Doty,[11] indicating that the material was largely ordered and of reasonably high molecular weight.

The spectra of the Hg^{++} complexes of the several nucleosides as observed in this Laboratory[12] are displayed in Fig. 3. The spectra were all taken in acid solutions containing excess Hg^{++} ion, and are, we believe, due to 1:1 mercury-base complexes. A brief further discussion of their formulas and structures is given in the Discussion section.

Hydrogen-Ion Release Experiments.—The object of these experiments was to determine whether H^+ ions are released when Hg^{++} ions react with DNA. Assuming that the Hg^{++} is bound by an unshared electron pair on a nitrogen atom, the qualitative question is whether the reactions are to

(11) J. Marmur and P. Doty, Nature, **183**, 1427 (1959).

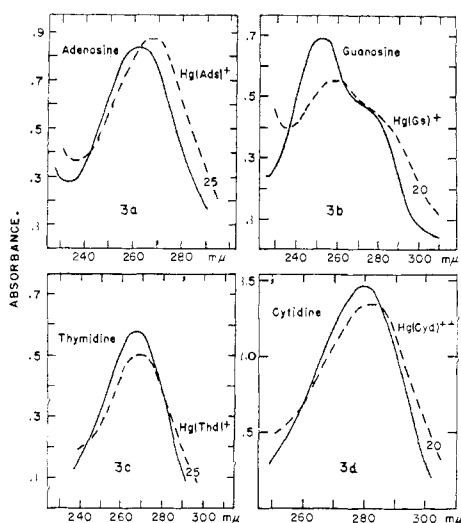(12) R. Ferreira, E. Ben-Zvi and N. Davidson, unpublished results.

Fig. 3.—Plot of absorbance ($= \log(I_0/I)$) of some purine and pyrimidine nucleosides in the presence of $Hg(ClO_4)_2$. The values of r = moles of Hg^{II}/moles of base are shown. In all cases $\mu = 0.10\ M$ ($NaClO_4$). (3a) Adenosine (Ads) ($6.0 \times 10^{-5}\ F$), pH 2.40; the formation constant[12] indicates that the second curve ($r = 25$) shows 70% of the adenosine complexed as $Hg(Ads)^+$ (3b). Guanosine (Gs) (5.0×10^{-5} F), pH 3.0; at $r = 20$, 75% of the nucleoside is present as $Hg(Gs)^+$ (3c). Thymidine (Thd) ($1.20 \times 10^{-4}\ F$), pH 2.4; at $r = 25$, the thymidine is completely complexed as $Hg(Thd)^+$. (3d) Cytidine (Cyd) ($1.10 \times 10^{-4}\ F$), pH 2.0; no quantitative equilibrium data are available, but we suspect that nucleoside is completely complexed in the form of $Hg(Cyd)^{++}$ at $r = 20$.

be described as

$$R_3N: + Hg^{++} \longrightarrow R_3N:Hg^{++}$$

or as

$$R_2NH + Hg^{++} \longrightarrow R_2N:Hg^+ + H^+$$
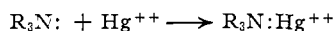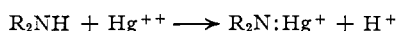
The experiments were a kind of pH-stat titration. They were performed either on a macro or a micro scale by adding a small amount of a Hg^{II} solution, either $HgCl_2$ or $Hg(ClO_4)_2$ (in the latter case with added $HClO_4$ for which corrections were made) to an unbuffered DNA solution. The pH decreased. The amount of NaOH required to bring the pH back to its initial value then was measured. The ratio of the moles of NaOH added to the moles of Hg^{II} added was then $\Delta H^+/\Delta Hg^{++}$, or the number of protons released per mercury added at the given value of r.

All solutions were at an ionic strength of 0.1 M ($NaClO_4$). (In pH titrations in perchlorate solutions, we use a 0.5 M $NaNO_3$ salt bridge between the Beckman saturated KCl, calomel electrode and the solution being titrated, because of the insolubility of $KClO_4$.) The DNA concentrations were $ca.$ 5 × 10^{-4} F in P. The pH's were adjusted to 5.55–5.60. In any one pH-stat titration, the pH could be returned to its initial value easily within ±0.02. Thus the uncertainty in H^+ is $\delta H^+/H^+ \approx 0.046$, or at pH 5.6, $\delta H^+ \approx 1.0 \times 10^{-7}$. With DNA concentrations of 5 × 10^{-4} F in phosphorus and increments, ΔHg^{++} of $ca.$ 2.5 × 10^{-5} M, the proton release was of the order of $\Delta H^+ = 5$–2.5×10^{-5} M. Thus, if there were no
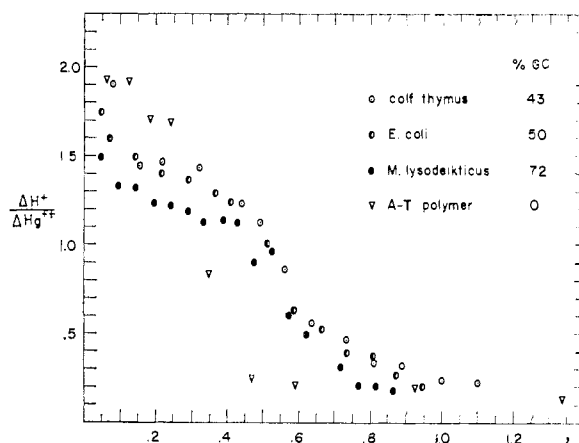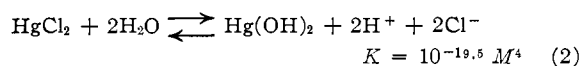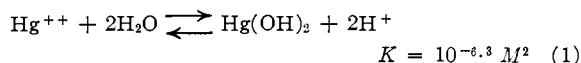

Fig. 4.—Plot of the number of protons released per mercury added ($\Delta H^+/\Delta Hg^{++}$) as function of r: $T = 24°$, 0.10 M $NaClO_4$, pH adjusted to 5.55–5.60, but constant for each experiment. DNA concentrations: calf thymus, 7.0 × 10^{-4} F in P; $E.\ coli$, 1.3 × 10^{-4} F; $M.\ lysodeikticus$. 4.6 × 10^{-4} F; A-T polymer, 1.0 × 10^{-4} F.

other sources of error, it should be possible to determine $\Delta H^+/\Delta Hg^{++}$ with an accuracy of 1% or better. Actually, reproducibility of about 10% was obtained. The results are displayed in Fig. 4. For calf thymus DNA and A-T polymer, the initial value of $\Delta H^+/\Delta Hg^{++}$ for the first addition of Hg^{++} is about 2.0. It is 1.8–2.0 for $E.\ coli$ DNA. For $M.\ lysodeikticus$ DNA, it appears to be about 1.5. In all cases as r increases, $\Delta H^+/\Delta Hg^{++}$ decreases. For the three natural DNA's, it is about 1.0 at $r = 0.5$, and there are over-all about 1.5 H^+ ions released per Hg^{++} added when r is increased from 0 to 0.5. For A-T polymer, the initial value of $\Delta H^+/\Delta Hg^{++} = 2$ drops rather abruptly to a value well below one in the range $0.2 < r < 0.3$.

In the range $0.5 \leqslant r \leqslant 1.0$, the values of $\Delta H^+/\Delta Hg^{++}$ decrease to about 0.2. These numbers are not directly significant, however, since they are the H^+ released per Hg^{II} added to the solution, not the H^+ released per Hg^{II} bound. Above $r = 0.5$, the binding is sufficiently weak that not all of the Hg^{II} added is bound.

We should also mention the equilibrium constants for the reactions[13]

$$Hg^{++} + 2H_2O \rightleftarrows Hg(OH)_2 + 2H^+$$
$$K = 10^{-6.3}\ M^2 \quad (1)$$

$$HgCl_2 + 2H_2O \rightleftarrows Hg(OH)_2 + 2H^+ + 2Cl^-$$
$$K = 10^{-19.5}\ M^4 \quad (2)$$

According to reaction 1, Hg^{++} is largely hydrolyzed for pH's > 3.2; in a titration with $Hg(ClO_4)_2$ at pH 5.6, we observed $\Delta H^+/\Delta Hg^{++} = 2$ as expected. Equilibrium 2 predicts that at pH 5.6, $[Hg(OH)_2]/[HgCl_2] = 5 \times 10^{-8}/[Cl^-]^2$. For $[Cl^-] > 3 \times 10^{-4}\ M$, the extent of hydrolysis is small. The observed pH-stat titration curve of $HgCl_2$ was in agreement with calculations based
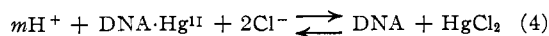
(13) Data from J. Bjerrum, G. Schwarzenbach and L. G. Sillén, "Stability Constants of Metal Ion Complexes; Part I, Organic Ligands; Part II, Inorganic Ligands," The Chemical Society, London, I (1957), II (1958).

on Eq 2   In the DNA titrations, the binding is essentially complete up to $r = 0.5$, and, as we shall show later, chloride ions are released when $HgCl_2$ is added and Hg^{++} binds to the DNA.   Thus when $r = 0.5$, $(Cl^-) = 5 \times 10^{-4}\ M$; with this Cl^- concentration, the additional $HgCl_2$ added as r is increased above 0.5 is not significantly hydrolyzed even though it is not all bound.

Chloride Ion Reversal Experiments.—Chloride ion is a good complexing agent for Hg^{II}.   The successive formation constants for the reactions $HgCl^{3-i}_{i-1} + Cl^- \rightleftarrows HgCl_i^{2-i}$, are: $\log K_1 = 6.74$, $\log K_2 = 6.48$, $\log K_3 = 0.85$, $\log K_4 = 1.00$ (in 0.5 M $NaClO_4$, 25°C).[13]   For Cl^- concentrations between $5 \times 10^{-4}\ M$ and $10^{-2}\ M$, for pH's < 5.7, the principal species in aqueous Hg^{II} solutions containing no other complexing ligands is $HgCl_2$. We shall need the over-all equilibrium constant for the reaction

$$Hg^{++} + 2Cl^- \rightleftarrows HgCl_2,$$

$$K_c = \frac{[HgCl_2]}{[Hg^{++}][Cl^-]^2} = 10^{13.22} \quad (3)$$
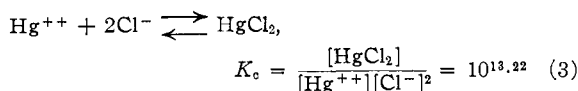
It already has been stated that the reaction

$$mH^+ + DNA\cdot Hg^{II} + 2Cl^- \rightleftarrows DNA + HgCl_2 \quad (4)$$

(where m is the number of protons released per Hg^{++} ion added to DNA) can be driven to the right by the addition of excess chloride ion. A quantitative study therefore offers the possibility of determining the equilibrium constants for the binding of Hg^{II} by DNA.[14]

For reasons to be expounded in the Discussion section, it was also desired to learn whether it was possible to add chloride ion to the complex without breaking the DNA–Hg^{II} bond, to give a DNA–Hg^{II}-Cl species.

Two kinds of experiments were performed. In one, an unbuffered DNA–Hg^{II} solution was titrated with chloride ion. As suggested by equation 4, the solution became less acid as the complex decomposed. The amount of acid required to restore the original pH was measured.

In the second kind of experiment, the change in optical absorbance with increasing chloride ion concentration at a fixed pH was measured at a wave length where the spectra of the DNA–Hg^{II} complex and of DNA are quite different.

The results of the two kinds of experiments for calf thymus DNA are displayed in Fig. 5. The solution was unbuffered at a pH of 5.60, with a DNA concentration of $7 \times 10^{-4}\ F$ in P, and with $r = 0.43$.   Because of the high concentration of DNA, the absorbance was measured at 300 mμ. The experiments on the variation of the absorbance with r (as in Fig. 2) showed that for $0 < r \leqslant 0.5$ the change in absorbance was a linear function of r. Therefore the vertical coördinate in the absorbance experiment is a measure of the ratio of bound mercury to bases in the DNA.   From the hydrogen-ion release experiments (Fig. 4), the relation between the total amount of H^+ released and r, the amount

(14) We did attempt, without success, to measure these equilibrium constants by a study of the potential of a Hg, Hg^{II} electrode in the presence of excess DNA.   The potentials were not steady and changed when the mercury surface was renewed, indicating perhaps adsorption of DNA on the electrode surface.
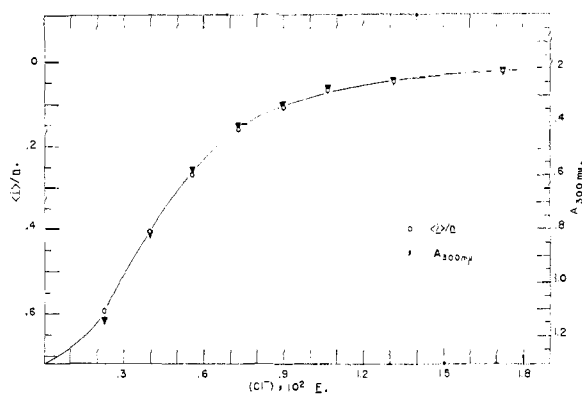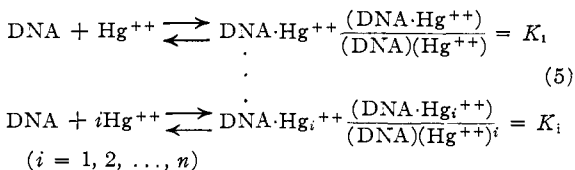

Fig. 5.—A (absorbance) and $<i>/n$ (where $<i>$ = no of moles of Hg^{II} bound to DNA and n = no. of DNA basepairs) changes accompanying the chloride back titration of mercurated calf thymus DNA, 7×10^{-4} F in P, $\mu = 0.10$ M $NaClO_4$, pH 5.60, unbuffered.   Absorbance taken at 300 mμ. The quantity $<i>/n$ was calculated from the amount of OH^- released when Cl^- is added to mercurated DNA, using the data in Fig. 4.

of Hg^{++} bound, is known for any particular DNA. It is evident from Fig. 5 that these two measures of the degree of dissociation of the complex are in good agreement.

In order to discuss the calculation of the binding constants for the DNA-Hg^{II} complex, we must first refine our notation and review the formalism for the binding of ions by a polymer molecule.

The quantity r previously introduced is the ratio of the number of Hg^{II} ions added to the number of bases present.   We are interested in the first complex for which $0 < r \leqslant 0.5$.   In the absence of additional added chloride, the amount of Hg^{II} bound is equal to the amount added for $0 < r \leqslant 0.5$. We consider that there are n binding sites per DNA molecule.   Since we are interested in the first complex, we take n equal to the number of base-pairs.   Thus, for $r = 0.5$, $r/n = 1.0$.

The complex with i Hg^{++} ions per DNA molecule, we now write as $DNA\cdot Hg_i^{++}$.   (For this particular complex, $r = i/2n$.)   The reactions for forming the various complexes can be written

$$DNA + Hg^{++} \rightleftarrows DNA\cdot Hg^{++} \frac{(DNA\cdot Hg^{++})}{(DNA)(Hg^{++})} = K_1$$
$$(5)$$

$$DNA + iHg^{++} \rightleftarrows DNA\cdot Hg_i^{++} \frac{(DNA\cdot Hg_i^{++})}{(DNA)(Hg^{++})^i} = K_i$$
$$(i = 1, 2, \ldots, n)$$

Under these circumstances, the average number of mercury ions per DNA molecule is given by

$$<i> = \frac{\sum_{i=0}^{n} i(DNA\cdot Hg_i^{++})}{\sum_{i=0}^{n} (DNA\cdot Hg_i^{++})} = \frac{\sum iK_i(Hg^{++})^i}{\sum K_i(Hg^{++})^i} \quad (6)$$

Define the function $\Xi(Hg^{++}) = \Sigma K_i(Hg^{++})^i$. (It is actually a kind of grand partition function.) We see from 6 that $\langle i \rangle = d \ln \Xi/d \ln (Hg^{++})$. The relation between $\langle i \rangle$ and the quantity r is

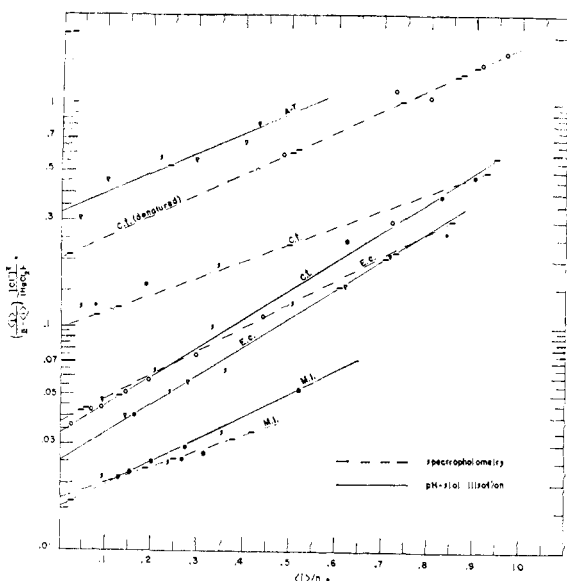$$\frac{r}{0.5} = \frac{\langle i \rangle}{n} = \frac{1}{n} \frac{d \ln \Xi}{d \ln (Hg^{++})} \quad (7)$$

Fig. 6.—Plot of log $<i>(Cl^-)^2/[n - <i>](HgCl_2)$ vs. $<i>/n$, for various DNA samples. Spectrophotometric experiments (dotted lines) were performed in a 0.01 F NaAc, HAc buffer at an initial pH of 5.7, with DNA concentrations of 1.7×10^{-4} F in P (calf thymus), 1.5×10^{-4} F ($E.\ coli$) and 1.7×10^{-4} F ($M.\ lysodeikticus$) with r's of ca. 0.5. pH-stat titration experiments (solid lines) carried out at pH 5.6, unbuffered, with DNA concentrations of 7.0×10^{-4} F (calf thymus), 1.3×10^{-4} F ($E.\ coli$), 4.6×10^{-4} F ($M.\ lysodeikticus$), and 1.0×10^{-4} F (A-T polymer). In all cases $\mu = 0.10$ M ($NaClO_4$). The abbreviations are as follows: c.t. (calf thymus DNA); E.c. ($E.\ coli$); M.l. $M.\ lysodeikticus$); A-T (A-T polymer).

If the n-sites on the molecule are all equivalent and if the probability of binding at one site is independent of whether or not there is any binding at adjacent sites

$$K_i = \frac{n!}{i!(n - i)!} K_0 \tag{8}$$

where K_0 is the intrinsic binding constant for a site. If 8 holds

$$\Xi = [1 + K_0(Hg^{++})]^n$$

and

$$\frac{(i)}{n} = \frac{K_0(Hg^{++})}{1 + K_0(Hg^{++})}$$

or

$$\frac{2r}{1 - 2r} = \frac{(i)}{n - (i)} = K_0(Hg^{++}) \tag{9}$$

This is, of course, the simple mass action law. If there is a repulsive interaction between occupied sites, such as an electrostatic repulsion between added Hg^{++} ions, the situation can be approximately represented by the equation[15]

$$\frac{(i)}{n - (i)} = K_0(Hg^{++}) e^{-w(i)/n} \tag{10}$$

where w is an interaction constant. In our experiments, the concentration of $HgCl_2$ is known from the initial amount of mercury added and the spectrophotometric or acidimetric measure of the amount still complexed by the DNA. Referring to eq. 3, $(Hg^{++}) = K_0(HgCl_2)/(Cl^-)^2$.

(15) I. Klotz in "The Proteins," Vol. I (H. Neurath, K. Bailey, ed.), Academic Press, Inc., New York, N. Y., 1953, Part B, p. 790.

Thus, an appropriate way to represent the data is

$$\log \frac{(i)(Cl^-)^2}{(n - \langle i \rangle)(HgCl_2)} = \log (K_0 K_c) -$$

$$2.3\, w\, \frac{(i)}{n} \tag{11}$$

The plot of the data for various DNA samples in this form is displayed in Fig. 6. As noted in the legend, most of the spectrophotometric experiments were done in a 0.01 F Ac^-, HAc buffer at an initial pH of 5.7 with a DNA concentration of 1.5×10^{-4} F in P and with initial r's of ca. 0.5. Thus, when the complex was almost completely dissociated, the hydrogen ion concentration had probably decreased by about 10%, giving a slightly smaller slope to the plots than would be the case at a fixed pH. The salient conclusions of these experiments are:

(a) Equation 11 represents the data fairly well and the slope parameter w is about the same for the different DNA's.

(b) The strength of the binding of (Hg^{++}) ion by different DNA's decreases in the order: AT polymer, heat-denatured calf-thymus DNA, native calf thymus, $E.\ coli$ and $M.\ lysodeikticus$.

(c) The acidimetric and the spectrophotometric experiments are in reasonably good agreement. (The greatest discrepancy is for thymus DNA at low values of $(i)/n$, where the experimental errors are greatest. The spectral and acidimetric results for this same DNA for an experiment under slightly different conditions (Fig. 5) are in good agreement.)

Sedimentation Experiment.—One set of ultracentrifuge velocity sedimentation runs was made in 0.1 M $NaClO_4$, 0.01 F NaAc buffer at a pH of 5.7, with a DNA concentration of 70 μg. $ml.^{-1}$ ($ca.$ 2.3×10^{-4} F in P) for native and heat-denatured samples and with $r = 0.0, 0.5$, and 1.2. The results are displayed in Table I.

TABLE I

SEDIMENTATION COEFFICIENTS OF MERCURATED DNA[a,b]

	Undenatured		Denatured	
r	$S_{w,20^o}$, sec.	$[\eta]$, ml. $\mu g.^{-1}$	$S_{w,20^o}$, sec.	$[\eta]$, ml. $\mu g.^{-1}$
0.0	18.8	0.0072	21.2	0.025
0.5	47.6	.0017	43.4	0.004
1.2	62.3	.0007	60.8	

[a] We are grateful to Mrs. Janet Morris for performing the sedimentation runs. [b] DNA concentration, 70 μg. $ml.^{-1}$.

Empirical or semiempirical methods of interpreting the sedimentation velocity of DNA preparations that are either "coils" or "rods" are available.[16] These interpretations are based on the extrapolated value of the sedimentation coefficient at zero DNA concentration, whereas our measurements were made at a rather high DNA concentration. Furthermore, the shape of the mercurated samples is unknown. We therefore believe that an attempt to interpret the data of Table I in terms of molecular weights is unwarranted and offer the results only as empirical items of information.

This is a convenient point to note that exact information as to the molecular weight and possible aggregation of the DNA-Hg^{II} complex

(16) P. Doty, J. Marmur, J. Eigner and C. Schildkraut, Proc. Natl. Acad. Sci. U. S., 46, 461 (1960).

is not available. Katz[7] concluded, from light-scattering measurements, that there is partial aggregation to dimers. For example, in a solution containing ca. 100 μg. ml.$^{-1}$ of DNA (ca. 3×10^{-3} F in DNA phosphorus) with an r of 0.64 Hg[II] bound per base (estimated by equilibrium dialysis experiments), light scattering was interpreted to indicate that 50% of the DNA molecules had associated to form dimers. The quantitative interpretation has been criticized by Thomas,[8] on the grounds that Katz did not consider the effect of the added Hg^{++} ions on the (dn/dc) of the DNA solute molecules. In addition, the reliability of the usual light-scattering measurements for molecular weight determinations of high molecular weight DNA's is open to some question.[16,17]

We conclude then that both the sedimentation and the light-scattering measurements indicate that there has been no gross polymerization of DNA to colloidal aggregates but that exact molecular weight data are not available.

Heat Denaturation Experiment.—The heat denaturation of DNA has been studied extensively (for references, see ref. 16). The so-called "melting temperature," T_m, is defined as the temperature at which 1 hr. heating will reduce the intrinsic viscosity, measured at 25° after rapid cooling, to one-half the original value. The melting range for a "good" DNA is usually rather narrow. The effect of Hg^{++} binding on the heat denaturation was studied by heating samples in 0.1 M NaClO$_4$, 0.01 F acetate buffer, pH 5.7, for 1 hr., cooling rapidly, decomposing the DNA·Hg[II] complex with NaCl and measuring the viscosity. As already noted, HgCl$_2$ in the presence of excess NaCl does not affect the viscosity of native unheated DNA. The observed T_m's for calf thymus DNA were: 85° (no HgCl$_2$); 80°, r = 0.5; 78°, r = 1.0. We have not investigated the effect of temperature on the Hg[II] binding, but it is probable that the complex exists at 80° and that it slightly decreases the denaturation temperature. The transitions were equally sharp with and without mercury.
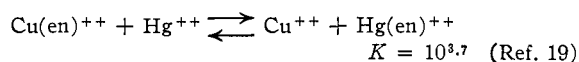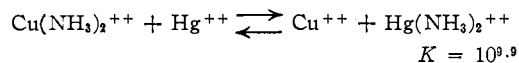
Discussion

It should be said at the outset that we are not able to propose a structural formula for the DNA·Hg[II] complex which is consistent with all of the facts presented in the previous sections. All that we are able to do is to enumerate some of the plausible possibilities and to point out the way in which they fail to explain the data.
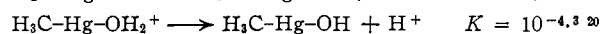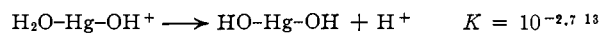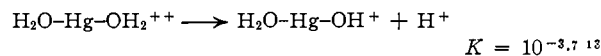
In order to outline these "plausible" possibilities, we begin by reviewing some general characteristics of mercury(II) complexes. Mercury(II) forms strong complexes by bonds with basic nitrogen atoms, sulfhydryl groups, Cl$^-$, Br$^-$, I$^-$ and CN$^-$. It forms relatively weak complexes by bonds to oxygen atoms. Mercury(II) tends to form two strong bonds and to have a much weaker binding constant for a third and a fourth ligand. Thus, as already stated, the successive binding constants for Cl$^-$ are: log K_1 = 6.74, log K_2 = 6.48, log K_3 = 0.85, log K_4 = 1.00. The successive

(17) J. A. V. Butler, D. J. R. Laurence, A. B. Robins and K. V. Shooter. *Proc. Roy. Soc. (London)*, **A250**, 1 (1959).

binding constants for NH$_3$ are: log K_1 = 8.8, log K_2 = 8.7, log K_3 = 1.00, log K_4 = 0.78.[13]

Mercury(II) complexes with two ligands tend to have a linear X–Hg–X configuration.[18] Consequently, compared to other complexing metals with octahedral or square configurations, mercury(II) has relatively less tendency to form chelate compounds. This is evidenced by comparing the equilibrium constants for two reactions, each of which involves the competition between Hg^{++} and Cu^{++} for two metal-nitrogen bonds (en is ethylenediamine).

$$Cu(NH_3)_2{}^{++} + Hg^{++} \rightleftarrows Cu^{++} + Hg(NH_3)_2{}^{++}$$
$$K = 10^{9.9}$$

$$Cu(en)^{++} + Hg^{++} \rightleftarrows Cu^{++} + Hg(en)^{++}$$
$$K = 10^{3.7} \quad (Ref. 19)$$

Another important fact about Hg^{++} is its tendency to hydrolyze. Some illustrative equilibria are

$$H_2O-Hg-OH_2{}^{++} \longrightarrow H_2O-Hg-OH^+ + H^+$$
$$K = 10^{-3.7}\ {}^{13}$$

$$H_2O-Hg-OH^+ \longrightarrow HO-Hg-OH + H^+ \quad K = 10^{-2.7}\ {}^{13}$$

$$H_3C-Hg-OH_2{}^+ \longrightarrow H_3C-Hg-OH + H^+ \quad K = 10^{-4.3}\ {}^{20}$$

Thus, above pH 3, Hg^{++} is largely hydrolyzed to Hg(OH)$_2$ and above pH 4.3, H$_3$C–Hg$^+$ is hydrolyzed to H$_3$C–Hg–OH.

We now briefly review our present knowledge of the Hg[II]-nucleoside complexes.[12]

(a) Let Ads represent neutral adenosine. With excess Ads, there is a complex Hg(Ads)$_2{}^{++}$.

(b) With excess Hg^{++}, there is a 1:1 complex.

(c) There is a loss of a proton on forming the 1:1 complex, which has the formula Hg(Ads)$^+$ rather than Hg(Ads)$^{++}$, even at pH 2.

Items a and b are typical and expected; item c is unexpected and difficult to explain. The structure of adenosine is (α) below
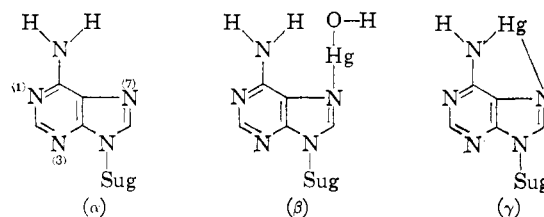
It would be plausible to expect mercury to form a nitrogen bond either at N-1, N-3 or N-7. We might attribute the loss of a proton in the 1:1 complex as being due to the hydrolysis to form a Hg–OH as in structure (β) above. This is not very plausible, however, in view of the pK's quoted for the hydrolysis of Hg[II] ions. Structure (γ) could explain the loss of a proton, but it is stereochemically unreasonable. Another unlikely possibility is that there is a chelate, with the mercury attached to N-3 and to a sugar oxygen. Thus, we regard the

(18) For actual structural data bearing on this point, see A. F. Wells, "Structural Inorganic Chemistry," Oxford University Press, London, 1950, pp. 631–634; see also, W. N. Lipscomb, *Annals New York Acad. Sci.*, **65**, 427 (1957).

(19) Data from ref. 13 and from J. I. Watters and J. G. Mason. *J. Am. Chem. Soc.*, **78**, 285 (1956).

(20) W. L. Hughes, *Annals New York Acad. Sci.*, **65**, 454 (1957).

	GC:AT ratio	λ_{max} $(r = 0)$	ϵ_{max}	Isosbestic λ $(0 < r < 0.5)$	λ_{max} $(r = 0.5)$	$\frac{\epsilon_{max}(r = 0.5)}{\epsilon_{max}(r = 0)}$
Calf thymus	0.75	258	6.60×10^3	262.5	269	1.016
E. coli	1.0	258	6.70×10^3	262.0	268	1.021
M. lyso.	2.6	257	6.92×10^3	261.5	269	0.99

structure of the $Hg(Ads)^+$ complex as unknown and puzzling.

(d) There is a 1:1 guanosine complex, which also has the charge of plus one. This is not difficult to understand since neutral guanosine has an ionizable N–H bond at N-1.

(e) The 1:1 thymidine complex evidently is formed according to the "reasonable" reaction
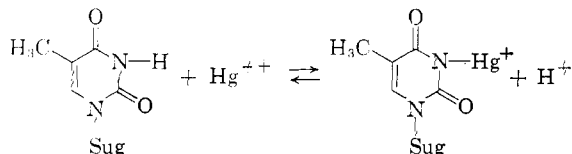
The formula of the cytidine complex is not yet known. Some spectra are presented in Fig. 3.

The facts about the DNA–Hg^{II} complex (for the case $0 < r \leqslant 0.5$, for which we have made the most measurements) which seems to us to be pertinent to the discussion of the structure of the complex are

(f) Not only is the addition of Hg^{II} reversible, so that native DNA can be recovered by removing the Hg^{II}, but there is a hypochromic effect for the native DNA·Hg^{II} complex as compared to denatured DNA·Hg^{II} complex.

Item (f) indicates that in spite of the decrease in viscosity on adding Hg^{II}, there is probably a very considerable degree of order in the base packing in the native DNA·Hg^{II} complex.

(g) The "first complex" with an isosbestic point at ca. 262 mμ forms until there is one mercury per base pair, irrespective of the GC:AT ratio of the DNA.

(h) The spectral shift on adding 0.5 Hg^{II} per base is similar for the various natural DNA's, but it is different for A–T polymer. However, as is evident in the data in Table II, the spectral shift for native DNA's cannot be attributed to an interaction with the GC pairs only.

(i) Pour-El and Dekker[9] have reported the spectral shifts on adding HgCl$_2$ ($r = 2.0$) to apyrimidinic acid and apurinic acid. The spectral shifts in the former case are rather like the shifts in DNA; the effects on apurinic acid are much less marked.

(j) The spectral shifts on adding Hg^{II} to DNA are qualitatively similar to those for the Hg^{II}-adenosine and Hg^{II}-cytidine complexes and different from the changes for Hg^{II}-guanosine and Hg^{II}-thymidine.

(k) The initial value of $\Delta H^+/\Delta Hg^{++}$ is close to 2 for $r = 0$ and gradually decreases to 1 as $r \rightarrow 0.5$, except for A–T polymer where $\Delta H^+/\Delta Hg^{++}$ decreases rather markedly around $r = 0.25$.

(l) The acidimetric and spectrophotometric measurements for the dissociation of the complex as chloride is added are in agreement.

(m) AT-rich DNA binds Hg^{++} more strongly than does GC-rich DNA.

We are unable to explain the values of $\Delta H^+/\Delta Hg^{++}$ of 1.8–2. If the mercury(II) ions were to add on to nitrogens not involved in the Watson–Crick hydrogen bond systems, such as N-3 or N-7 of adenosine and guanosine, it is quite probable that at pH 5.6, the mercury would have an OH on its other side, as in structure β for the adenosine-Hg complex above. This would give $\Delta H^+/\Delta Hg^{++}$ = 1.0. Furthermore, one would expect that the reaction

$$\equiv N:Hg{-}OH + Cl^- \longrightarrow \equiv N:Hg{-}Cl + OH^-$$
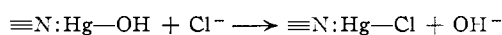
would occur, consuming H$^+$ but not significantly affecting the spectrum, in contradiction to item 1.

Suppose the Hg^{++} goes between two bases that were previously bonded by the Watson–Crick hydrogen bonds. A reasonable structure for an A-T pair would be (δ) below

(δ)
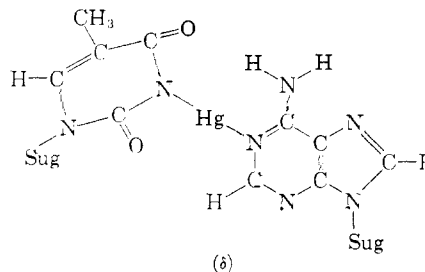
(The hydrogen bond between the amino NH of adenine and the carbonyl oxygen of thymine would probably be broken because of the greater length of the N–Hg–N system.) This model predicts $\Delta H^+/\Delta Hg^{++} = 1.0$.

A structure that predicts $\Delta H^+/\Delta Hg^{++} = 2$ would be an N–Hg–N bond between N-3 of thymidine and the amino nitrogen of adenosine, with loss of an amino proton as well as a thymine proton. This is not very plausible but it is conceivable. A similar structure for a GC pair is possible.

Neither this hypothesis nor any other that we can advance explains the spectroscopic differences between AT polymer and the natural DNA's.

All of our discussion has been based on the assumption that the Hg^{++} complexes are of the conventional type for the interaction of a heterocyclic nitrogen base with a Hg^{++} ion, involving a bond between an unshared sigma electron pair on the nitrogen and the mercury ion, with the mercury atom in the plane of the ring. Mercury(II) also forms π-complexes with olefins,[21] and it may be that the DNA·Hg^{II} complexes involve the interaction of a Hg^{II} ion with two bases that are vertically over each other in one of the strands of the double helix. Since each strand of AT polymer has the regular alternating structure, -A-T-A-T-,

(21) S. Ahrland, J. Chatt and N. R. Davies, Quart. Rev., 12, 265 (1958).

the natural DNA's where many other sequences occur could then be quite different from AT polymer as regards the Hg^{II} complex.

Thus, we are unable to propose a structure which explains the properties of the $DNA \cdot Hg^{II}$ complex as observed by us and by previous investigators.

We wish to add several other comments about the properties of the complex.

The experiments reported here seem to us to show quite conclusively that the complex involves the addition of Hg^{++} to the bases and not $HgCl_2$. Similar spectral changes and similar values of $\Delta H^+/\Delta Hg^{++}$ were observed for adding $Hg(ClO_4)_2$ and $HgCl_2$ to DNA. The data in Fig. 5 show that the complex is dissociated by added chloride concentrations of the order of $5 \times 10^{-3} M$. If the complex were actually $DNA \cdot HgCl_2$, the chloride reversal would be due to the reaction

$$DNA \cdot HgCl_2 + Cl^- \rightleftharpoons DNA + HgCl_3^-$$
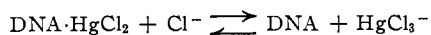
but since the equilibrium constant of the reaction $HgCl_2 + Cl^- \rightleftharpoons HgCl_3^-$ is $\log K_3 = 0.57$, this reaction could not be important with $[Cl^-] = 5 \times 10^{-3} M$. Although the spectral changes are similar for Hg^{++} and $HgCl_2$ for $r > 0.5$, our conclusion is most certain for $r < 0.5$. Pour-El and Dekker[9] did a qualitative analysis on a precipitate obtained by mixing DNA and $HgCl_2$ at high concentrations. They found both chloride and mercury to be present and concluded that a $DNA \cdot HgCl_2$ complex was formed. It appears to us that our experiments are more quantitative and more reliable, at least as regards the properties of the soluble complex in dilute solution and for $r \leqslant 0.5$.

In view of the evidence already cited that there is considerable order in the base packing in the complex, we now consider the significance of the decrease in viscosity on complex formation.

There is considerable evidence that native DNA in solution is not a single rigid rod but a slightly coiled or occasionally kinked rod. For samples of calf thymus DNA of molecular weight of ca. 6×10^6, the "hydrodynamic length" is about 5500 Å. whereas the contour length is about 31,000 Å.[22] Applying the random walk formula, this suggests that the original molecule contains about $(31,000/5500)^2 = 30$ kinks. The intrinsic viscosity $[\eta]$ of a rod-like molecule of length L is approximately proportional to $L^{1.7}$. If this molecule were divided by $N - 1$ kinks into N segments and if we make the simplifying assumption that each segment is hydrodynamically independent, $[\eta]$ would be proportional to $N(L/N)^{1.7} = L^{1.7}/N^{0.7}$. On this

(22) M. E. Reichmann, S. A. Rice, C. A. Thomas and P. Doty, J. Am. Chem. Soc., **76**, 3047, 6421 (1954).

basis the decrease in $[\eta]$ by a factor of four on mercuration corresponds to the division of every segment in the unmercurated molecule into seven new segments. While this argument is obviously very approximate, it does suggest that the decrease in viscosity on mercuration does not necessarily imply complete collapse to a disordered structure.

Finally, we remark that the difference in binding of Hg^{++} by native and denatured DNA's and between different DNA's (Fig. 6) may be useful for the fractionation of DNA samples.

Experimental

(23) J. Marmur, private communication.

(24) O. T. Avery, C. M. MacLeod and M. McCarty, J. Exptl. Med., **79**, 137 (1944).

(25) N. Schneider, as quoted in A. Holtzer, H. Benoit and P. Doty J. Phys. Chem., **58**, 624 (1954).