I. COMPLEXES OF MERCURY (I) WITH POLYPHOSPHATE AND DICARBOXYLATE ANIONS AND MERCURY (II) PYROPHOSPHATE COMPLEXES

II. THE INTERACTION OF MERCURIC CHLORIDE WITH DEOXYRIBONUCLEIC ACID

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ABSTRACT

I. It has been discovered that mercurous mercury forms complexes with pyrophosphate, tripolyphosphate, oxalate, α-dimethylmalonate, and succinate. These complexes are stable towards disproportionation to mercury (II) complexes and mercury. If L^{-q} is the anion, the principal complexes are $Hg_2L_2^{-2q+2}$ and $Hg_2(OH)L^{-q+1}$. The formation constants were determined from the potential of a mercury-mercurous electrode in ligand solutions. Theory and experiment agree that mercurous complexes of ligands (such as NH_3 and CN^-) which form strong covalent bonds to mercury are unstable towards disproportionation to give mercuric complexes but "ionic" chelating ligands can form stable mercurous complexes.

The mercury (II) pyrophosphate complex was studied from the potential of a Pt electrode in Hg_2^I, Hg^{II}, pyrophosphate solutions at pH 7-10. The principal species is $Hg(OH)(P_2O_7)^{-3}$, with a formation constant of $(2.8\pm0.6)10^{17}\underline{M}^{-2}$.

II. The interaction of mercury (II) with DNA was studied. The formation of the DNA-Hg^{II} complex is reversible according to spectrophotometric, viscometric, and biological criteria. Based on the ultraviolet absorption spectra and on the number of protons liberated from DNA during complexation, the binding sites of Hg^{II} to DNA are discussed.

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COMPLEXES OF MERCURY (I) WITH POLYPHOSPHATE AND DICARBOXYLATE ANIONS AND MERCURY (II) PYROPHOSPHATE COMPLEXES

INTRODUCTION

The equilibrium constant for the formation of mercurous ion from elementary mercury and mercuric ion is 130 in 0.5 M $NaClO_4$.[1] The equilibrium is readily reversible. When a

$$Hg + Hg^{++} = Hg_2^{++} \qquad K = (Hg_2^{++})/(Hg^{++}) = 130 \qquad (1)$$

complexing ligand is added to a mercurous solution, the usual reaction that occurs is disproportionation of the mercurous ion to give elementary mercury and a complexed mercuric ion. This occurs, for example, with the complexing ligands, CN^- and NH_3. It is due to the relatively greater stability of the mercuric complexes. The same situation occurs for many insoluble compounds. Thus, mercurous ion is unstable in basic solutions and in the presence of sulfide ion. Compounds such as "mercurous sulfide" or "mercurous oxide" reported in the past have been shown to be a mixture of mercury and the corresponding mercuric compound.[2,3]

The general impression conveyed by textbooks and by the chemical literature is that there are no known stable complexes of mercurous ion. Sillén and coworkers have suggested, on the basis of potentiometric evidence, that there are weak

complexes of Hg_2^{++} formed by nitrate, sulfate and perchlorate anions, with formation constants of: 2.5 $\underline{M}^{-1}$ ($Hg_2NO_3^+$), 0.5$\underline{M}^{-2}$ ($Hg_2(NO_3)_2$), 20 $\underline{M}^{-1}$ (Hg_2SO_4), 250 $\underline{M}^{-2}$ ($Hg_2(SO_4)_2^{-2}$) and 0.9$\underline{M}^{-1}$ ($Hg_2ClO_4^+$).[1,4] This presumably is mainly ion-pair association. It is also possible that the assumption of constant activity coefficients at constant ionic strengths is not sufficiently reliable to enable one to identify such weak complexes with certainty by potentiometric experiments.

However some time ago, Stromeyer[5] and then Brand[6] reported that when sodium pyrophosphate solution is added to a mercurous solution, a white precipitate forms and then redissolves in excess of the reagent, which suggests the formation of a strong, stable complex.

We have confirmed and extended these observations and have now found that mercurous ion forms stable complexes with pyrophosphate (Py^{-4}), tripolyphosphate (Tp^{-5}), oxalate (Ox^{-2}), α-dimethylmalonate (Ma^{-2}) and succinate (Su^{-2}) anions. For all these cases, the presence of Hg^I in the solutions can be shown by the quantitative precipitation of Hg_2Cl_2 upon addition of NaCl solution, and also by the characteristic ultraviolet spectrum of mercurous (see later). The Hg^I complexes can be formed by mixing Hg^{II}-ligand solutions with elementary mercury, showing that the Hg^I complexes are stable to disproportionation.[7]

EXPERIMENTAL

Materials. - Mercuric nitrate was prepared by dissolving HgO in HNO_3 and was standardized by titration against KCNS with ferric ion as indicator.[8]

Mercurous nitrate solutions were prepared by shaking together for one hour Hg, $Hg_2(NO_3)_2$ and HNO_3. The reagent was standardized gravimetrically by precipitation of the chloride and also by the bromophenol-blue method.[9]

Baker's reagent grade $Na_4P_2O_7 \cdot 10H_2O$ was used. Sodium tripolyphosphate, $Na_5P_3O_{10} \cdot 6H_2O$, was obtained from technical grade sodium tripolyphosphate as described by Watters.[10]

Allied Chemical & Dye Corporation's $K_2C_2O_4 \cdot H_2O$ was used as a source of potassium oxalate. Potassium malonate was prepared from the technical malonic acid as described by Bailar.[11] α-dimethylmalonic acid from K. & K. (Kuthe and Kuthe) laboratories was used as supplied. Matheson's reagent grade sodium succinate was recrystallized twice from water.

Potentiometric Method. - The cell was made up with a Beckman saturated calomel electrode, a J-type mercury electrode in which the mercury surface could be renewed by overflow, and a salt bridge (0.75 M $NaNO_3$). There were provisions for titrating in reagents and for maintaining a nitrogen atmosphere. A magnetic stirrer was used. The cell was in a water-bath at 27.4 ± 0.1°C. The emf's were measured with a Leeds and Northrup type K-2 potentiometer and a 0.01 μamp. per mm. galvanometer;

pH measurements were made with a Beckman GS pH Meter and a Beckman General Purpose Glass Electrode. In all cases, a stable potential was established almost instantly after addition of a reagent.

All optical measurements were made with a Cary Model 11 Spectrophotometer at room temperature. A hydrogen lamp and quartz cells with a path length of 1.00 cm. were used.

The potentiometric measurements were made at 27.4±0.1°C and in most cases, at an ionic strength of 0.75 M adjusted with $NaNO_3$. Due to low solubilities of mercurous oxalate and mercurous succinate salts, an ionic strength of 2.5 M, adjusted with $NaNO_3$, was used in both cases.

RESULTS

Analysis. - The experimental data indicate the necessity of considering the following equilibria:

$$Hg_2^{++} + 2L^{-q} = Hg_2L_2^{-2q+2} \qquad K_2 \qquad (2)$$

$$Hg_2^{++} + OH^- + L^{-q} = Hg_2(OH)L^{-q+1} \qquad K_1 \qquad (3)$$

The potential of the mercury electrode with respect to the reference electrode is given by

$$E = E^\circ + 29.7 \log (Hg_2^{++}) \qquad (4)$$

with E° vs. S.C.E. = 539 mv at 27.4°C.

From stoichiometric considerations we obtain for the total mercurous ion concentration*

$$(\Sigma\ Hg_2^I) = (Hg_2^{++}) + (Hg_2(OH)L^{-q+1}) + (Hg_2L_2^{-2q+2}) \qquad (5)$$

and with the equations for the formation constants, the following relation results:

$$\frac{(\Sigma\ Hg_2^I)}{(Hg_2^{++})} - 1 = \underline{K}_1(OH^-)(L^{-q}) + \underline{K}_2(L^{-q})^2 \qquad (6)$$

$$\left(\frac{(\Sigma\ Hg_2^I)}{(Hg_2^{++})} - 1\right) \Big/ (L^{-q}) = \underline{K}_1(OH^-) + \underline{K}_2(L^{-q}) \qquad (7)$$

The total mercurous concentration, $(\Sigma\ Hg_2^I)$, is of course known. The free mercurous ion concentration, (Hg_2^{++}), is calculated from the observed potential by equation 4. Usually, the ligand concentration was much larger than the total mercurous concentration; where necessary, corrections for the amount of ligand complexed were made by a successive approximations procedure. Corrections for the hydrogen ion equilibria of the ligands were also made as discussed below.

Since the determination of formation constants involves the concentrations of unprotonated ligands as they exist in solution, effective pK's were measured by pH titration curves for the media used in the complexing experiments. The results

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* We should also include $Hg_2(OH)^+$ with a formation constant[12] of $10^9\ \underline{M}^{-1}$, but its concentration is negligible compared to $Hg_2(OH)\bar{L}$ and Hg_2L_2.

are displayed in Table I. Since the alkali metals form complexes with pyrophosphate and tripolyphosphate ions,[13] the values given in Table I differ from those determined in media where the supporting electrolyte contains tetraalkyl ammonium cations. The results in Table I, of course, are appropriate values for the analysis of our complexing data.

Table I

p$\underline{K}$'s of Ligands Studied

Pyrophosphate	$p\underline{K}_4$ = 8.00[a]	
	$p\underline{K}_3$ = 5.68[a]	
Tripolyphosphate	$p\underline{K}_5$ = 7.58[b]	
	$p\underline{K}_4$ = 5.29[b]	

	Oxalate[c]	α-dimethylmalonate	Succinate[c]
$p\underline{K}_2$	3.66	5.48	5.20
$p\underline{K}_1$	1.62	2.88	3.85

(a) $p\underline{K}_4$ = 8.93 and $p\underline{K}_3$ = 6.13 for μ = 1.0 $\underline{M}$ $(CH_3)_4NCl$.[13]
(b) $p\underline{K}_5$ = 8.81 and $p\underline{K}_4$ = 5.83 for μ = 1.0 $\underline{M}$ $(CH_3)_4NCl$.[10]
(c) μ = 2.5 $\underline{M}$ $NaNO_3$; all other data for 0.75 $\underline{M}$ $NaNO_3$.

Mercury (I) and Mercury (II) Pyrophosphate Complexes.- Figure 1 is a plot of the function on the left-hand side of equation 7 vs. molar concentration of $P_2O_7^{-4}$ at a given pH. (The concentration of $P_2O_7^{-4}$ was calculated from the total pyrophosphate concentration, using the acid constants of Table I, and making the necessary small corrections for the amount complexed). Equation 7 predicts an intercept of $\underline{K}_1(OH^-)$ and

Fig. 1

Plot of equation 7 at different pH's for the determination of $\underline{K}_1$ = intercept/(OH^-) and $\underline{K}_2$ = slope; $(\Sigma\ Hg_2^{++}) \simeq 1x10^{-5}$ - $1x10^{-4}$ $\underline{F}$; $(\Sigma\ Py) \simeq 0.004$ - 0.3 $\underline{F}$; Δ refers to the points obtained from the potentials either more positive or only slightly less positive than that of an Hg, HgO electrode. Note the change in horizontal scale for the upper half of the figure.

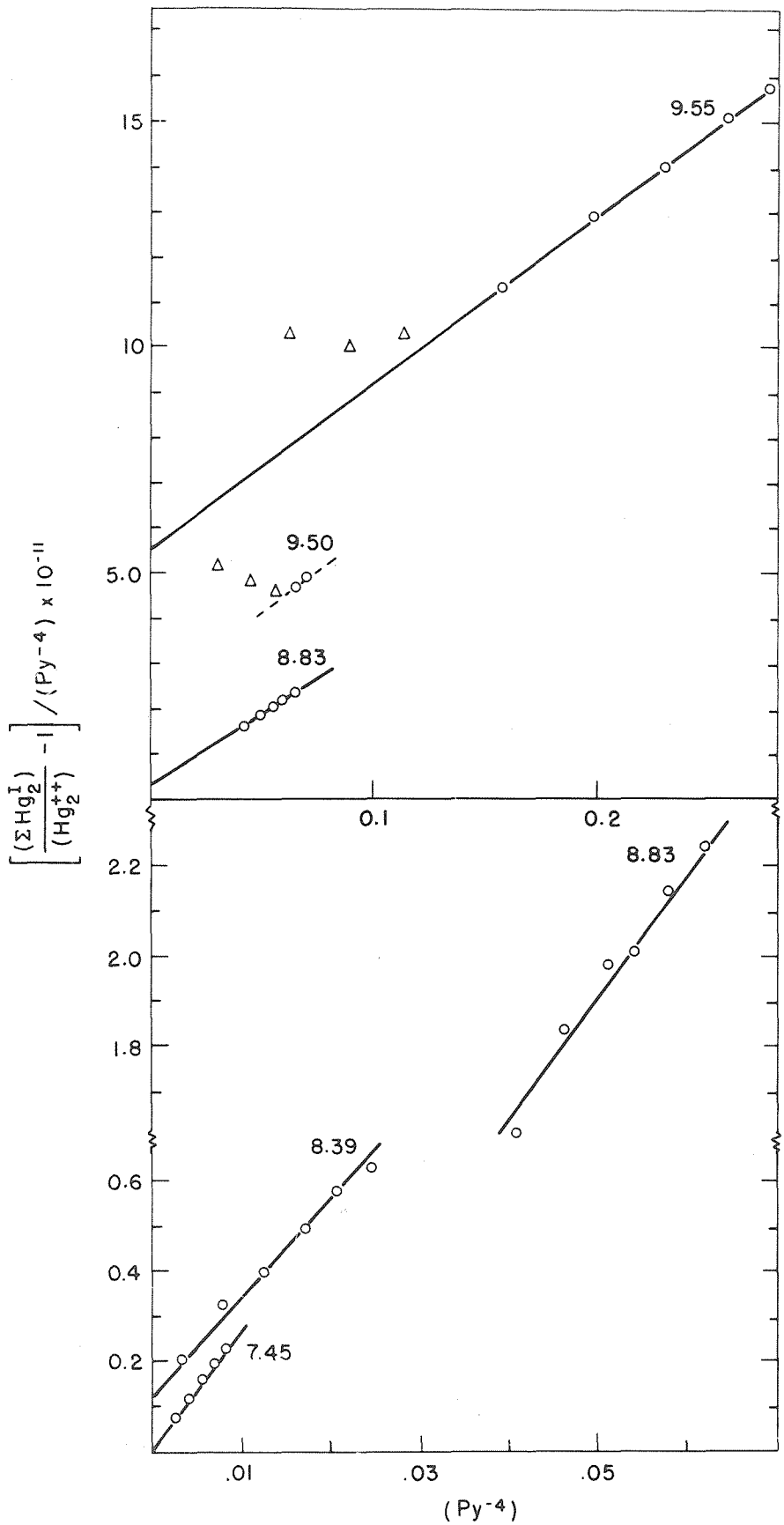

9.55
9.50
8.83
8.83
8.39
7.45
15
10
5.0
2.2
2.0
1.8
0.6
0.4
0.2
0.1
0.2
.01
.03
.05
(Py⁻⁴)
[(ΣHg₂ᴵ / (Hg₂⁺⁺)) - 1] / (Py⁻⁴) x 10⁻¹¹

Fig. 1

a slope of $\underline{K}_2$. At low pH (< 9), the intercepts increase with increasing pH and the slopes are constant, as expected from equation 7. However at high pH and low pyrophosphate concentrations, the plots curve upwards as the pyrophosphate concentration decreases. We believe that this behavior is due to the disproportionation of mercurous salts to mercuric oxide and mercury

$$Hg_2^{I} \rightarrow HgO + Hg$$

The standard potential of the Hg,HgO electrode is[14]

$$Hg + 2H_2O = (HgO)_c + 2H^+ + 2e^- \qquad \underline{E}^o = 931 \text{ mv}$$

$$\underline{E} \text{ (vs. S.C.E.)} = 690 + 59.5 \log (H^+) \qquad (8)$$

The solubility of HgO is $2.25 \times 10^{-4} \underline{M}$[14]; so, in solutions where the mercury concentration is less than $2.25 \times 10^{-4} \underline{M}$, mercury will be present in solution as HgO if the potential is more positive than that given by the equation

$$\underline{E} = 690 + 29.75 \log \frac{(\Sigma \text{ Hg})}{2.25 \times 10^{-4}} + 59.5 \log (H^+) \qquad (9)$$

The data in Fig. 1 which do not conform to equation 7 were all for potentials either more positive or only slightly less positive than calculated from equation 9. However, at high pyrophosphate concentrations at pH 9.55, the expected behavior from equation 7 with reasonable values of $\underline{K}_1$ and $\underline{K}_2$ is observed.

In summary of this point, the situation at high pH appears to be that at low pyrophosphate concentration, there is disproportionation according to the equation

$$Hg_2^{I}(OH)Py^{-3} + OH^- = HgO + Hg + Py^{-4} + H_2O \qquad (10)$$

and the observed potential is close to that of the Hg,HgO electrode. At higher Py concentration, reaction 10 proceeds to the left and the mercurous hydroxy pyrophosphate anion is present. It is remarkable that the mercuric oxide formed at low Py is not lost by adsorption on the walls and that reaction 10 is quantitatively reversed at high Py.

The results obtained for the slopes and intercepts, and hence for $\underline{K}_1$ and $\underline{K}_2$ of equation 7, are given in Table II. For reasons to be explained shortly, we now refer to $\underline{K}_1'$ rather than $\underline{K}_1$.

Table II

Slope ($\underline{K}_2$) and Intercept ($\underline{K}_1'/(OH^-)$) from Fig. 1

pH	$\underline{K}_1' \cdot 10^{-15}$ $\underline{M}^{-2}$	$\underline{K}_2 \cdot 10^{-12}$ $\underline{M}^{-2}$
7.12		2.70
7.45		2.84
8.34	5.95	2.10
8.39	5.90	1.95
8.83	7.20	2.80
8.90	6.90	(2.40)[a]
9.55	15.2	3.70[b]
ave.	$(6.5 \pm 0.7)10^{15}\underline{M}^{-2}$	$(2.4 \pm 0.6)10^{12}\underline{M}^{-2}$

(a) Only one pyrophosphate concentration, slope assumed.

(b) Slope and intercept from high pyrophosphate concentration only; results not included in averaging.

It is worthwhile to explicitly report that the Nernst

law behavior as regards Hg_2^{++} concentration was observed both at high and low pH (for points where the potentials showed that HgO was not present). These data are shown in Table III.

Table III

Validity of the Nernst Law

$(\Sigma\ Py) = 0.020\ \underline{F}$

	pH = 7.12		pH = 8.90	
$(\Sigma\ Hg_2^I)\cdot 10^5 / \underline{F}$	$\underline{E}$ (obs.) mv	$\underline{E}$ (calc.)	$\underline{E}$ (obs.) mv	$\underline{E}$ (calc.)
0.951	167.8		106.0	
1.89	176.5	176.7	115.2	114.9
3.84	186.5	185.8	124.6	124.0
5.75	192.3[a]	191.2	129.9[a]	129.2
9.35	198.8[a]	197.4	137.0[a]	135.5

(a) Corrections were made due to a slight change in pH.

Mercury (II) pyrophosphate complexes were studied from the potential of a Pt electrode in a Hg_2^{++}, Hg^{++} pyrophosphate solution.

The potential of the mercurous-mercuric couple with respect to the reference electrode is given by

$$\underline{E} = \underline{E}^{\circ} + 59.5 \log (Hg^{++})/(Hg_2^{++})^{1/2} \qquad (11)$$

with $\underline{E}^{\circ} = 637$ mv (vs. S.C.E.) at 27.4°C.

Substituting (Hg_2^{++}) as a function of $\underline{K}_1$ and $\underline{K}_2$, the following equation results:

$$\log(Hg^{++}) = \frac{E-E^{o}}{59.5} + \tfrac{1}{2}\log(\Sigma Hg_2^{I}) - \tfrac{1}{2}\log\left[K_1(OH^-)(Py^{-4}) + K_2(Py^{-4})^2\right]$$

The ratio $(\Sigma Hg^{II})/(Hg^{++})/(Py^{-4})(OH^-)$ gives fairly constant value at different pH's (Table IV), which indicates that the main species in these media is $Hg^{II}(OH)Py^{-3}$ with a formation constant of $K_{II} = 2.8 \times 10^{17}\ M^{-2}$. It is to be noted that the data were taken at pH's where the main mercurous species is $Hg_2^{I}Py_2^{-6}$, so the uncertainties about K_1 considered below do not affect the evaluation of K_{II}.

Table IV

Mercury (II) Pyrophosphate Complexes

pH	(ΣHg^{II})	(ΣPy)	$\frac{(\Sigma Hg^{II})/(Hg^{++})}{(Py^{-4})(OH^-)}$
7.62	1.0×10^{-4} F	$5\times10^{-3} - 3\times10^{-2}$ F	3.42×10^{17}
8.00	$5\times10^{-5} - 4\times10^{-4}$	2×10^{-2}	2.41×10^{17}
8.00	$5\times10^{-5} - 4\times10^{-4}$	9×10^{-3}	3.37×10^{17}
8.42	$2\times10^{-4} - 2\times10^{-3}$	5×10^{-3}	2.29×10^{17}

ave. $K_{II} = (2.8\pm0.6)10^{17} M^{-2}$

We may now inquire as to the possibility of the disproportionation reaction

$$Hg_2^{I}(OH)(Py)^{-3} = Hg^{II}(OH)(Py)^{-3} + Hg \qquad K_D \qquad (12)$$

From the equilibria

$$Hg^{++} + Hg = Hg_2^{++} \qquad K = 130$$

$$Hg^{II}(OH)Py^{-3} = Hg^{++} + OH^{-} + Py^{-4} \qquad \underline{K} = 1/\underline{K}_{II}$$

$$Hg_2^{++} + OH^{-} + Py^{-4} = Hg_2^{I}(OH)Py^{-3} \qquad \underline{K}_1$$

we calculate for equation 12, using for $\underline{K}_1$ the value of $\underline{K}_1'$ of Table II, $\underline{K}_D = 0.33$. This value is of the order of unity, indicating significant disproportionation of $Hg_2^{I}(OH)Py^{-3}$ to $Hg^{II}(OH)Py^{-3}$. Thus, the solutions used for determining the formation constant of $Hg_2^{I}(OH)Py^{-3}$ contained significant quantities of $Hg^{II}(OH)Py^{-3}$. It should be noted that the occurrence of this disproportionation reaction would not affect the functional dependence of the potential on Hg^{I}, Py and OH^{-} concentrations in the mercurous experiments. However, equation 7 would now become

$$\frac{\frac{(\Sigma Hg_2^{I})}{(Hg_2^{++})} - 1}{(Py^{-4})} = \underline{K}_1(1 + \underline{K}_D)(OH^{-}) + \underline{K}_2(Py^{-4}) \qquad (13)$$

Thus, the constant $\underline{K}_1'$ is $\underline{K}_1(1 + \underline{K}_D)$.

From the equations

$$\underline{K}_1' = 6.5 \times 10^{15} = \underline{K}_1(1 + \underline{K}_D)$$

$$\underline{K}_{II} = 2.8 \times 10^{17}$$

$$\underline{K}_D = \underline{K}_{II}/\underline{K}_1 \times 130 \text{ , we obtain}$$

as corrected values

$$\underline{K}_D = 0.49$$

$$\underline{K}_1 = 4.4(\pm 0.6) \times 10^{15}\ \underline{M}^{-2}.$$

However, the possibility remains that our data are quantitatively in error and that the complex we have identified as $Hg_2^{I}(OH)Py^{-3}$ is entirely $Hg^{II}(OH)Py^{-3}$. We believe that this possibility is eliminated by direct observations of the equilibrium of equation 12 by chemical analysis and ultraviolet spectrophotometry.

A Hg(II)-pyrophosphate solution ($(\Sigma Hg(II))=4.7x10^{-4}$ F, $(\Sigma Py) = 0.02$ F) at pH 9.4 was equilibrated with elementary mercury. The amount of Hg_2^{I} formed was analyzed by taking its characteristic ultraviolet spectrum and also by separating it in the form of Hg_2Cl_2. These analyses gave an average of $3.8x10^{-4}$ F for (ΣHg_2^{I}). Since the ratio $(Hg_2^{I}(OH)Py^{-3})/(Hg_2^{I}(Py)_2^{-6}) = 2.34$ at pH 9.4 and $(\Sigma Py) = 0.02$ F, the K_D from this experiment for equation 12 comes out 0.31 in good agreement with the calculated value from the potential measurements.

As shown in Fig. 2, there is a characteristic ultraviolet spectrum for mercury (I) which is just about the same for Hg_2^{++}, $Hg_2^{I}(Py)_2^{-6}$ and $Hg_2^{I}(OH)Py^{-3}$. The change in absorbance with pH is just as expected on the basis of the equilibrium constants derived previously, which also tends to confirm the existence of $Hg_2^{I}(OH)Py^{-3}$ (Fig. 3). It should also be mentioned that in the alkaline solutions air oxidation of $Hg_2^{I}(OH)Py^{-3}$ to $Hg^{II}(OH)Py^{-3}$ is rapid and difficult to avoid.

In summary, then, we believe the evidence strongly proves the existence of the complex $Hg_2^{I}(OH)Py^{-3}$, as well as

Fig. 2

Molar absorptivity, $\epsilon = (1/\ell c)\log_{10}(I_o/I)$, of several mercurous solutions. Conditions: $(\Sigma Hg_2^I) = 7.50 \times 10^{-5}$ F, $(\Sigma Py) = 0.020$ F, $\mu = 0.75$ F ($NaClO_4$). Using the equilibrium constants in the text, the curve labelled $Hg_2Py_2^{-6}$ at pH 7.70 is calculated to contain 90% $Hg_2Py_2^{-6}$ and 10% $Hg_2(OH)Py^{-3}$; the curve labelled $Hg_2(OH)Py^{-3}$ is calculated to contain 71% $Hg_2(OH)Py^{-3}$ and 29% $Hg_2Py_2^{-6}$. In agreement with these constants, the spectrum changes but slightly in the range 6.70-8.00. The curve labelled Hg_2^{++} was a $Hg_2(ClO_4)_2$ solution in 0.1 M $HClO_4$, 0.75 M $NaClO_4$. $Hg^{II}(OH)Py^{-3}$ curve obtained from a solution $(\Sigma Hg^{II}) = 3.0 \times 10^{-4}$ F and $(\Sigma Py) = 0.020$ F at pH 8.60; the Hg^{++} curve was a $Hg(ClO_4)_2$ solution, 0.5 M in $HClO_4$ and $\mu = 0.75$ M ($NaClO_4$).

Fig. 3

Plot of A (absorbance) $= \log_{10}(I_o/I)$ for $\lambda = 237$ mμ for mercurous pyrophosphate solutions at different pH's, vs. mole fraction of $Hg_2(OH)Py^{-3}$ calculated from the equilibrium constants in the text. This graph shows that the change in A with pH is consistent with K_1 and K_2 determined potentiometrically. The pH is given next to the points.

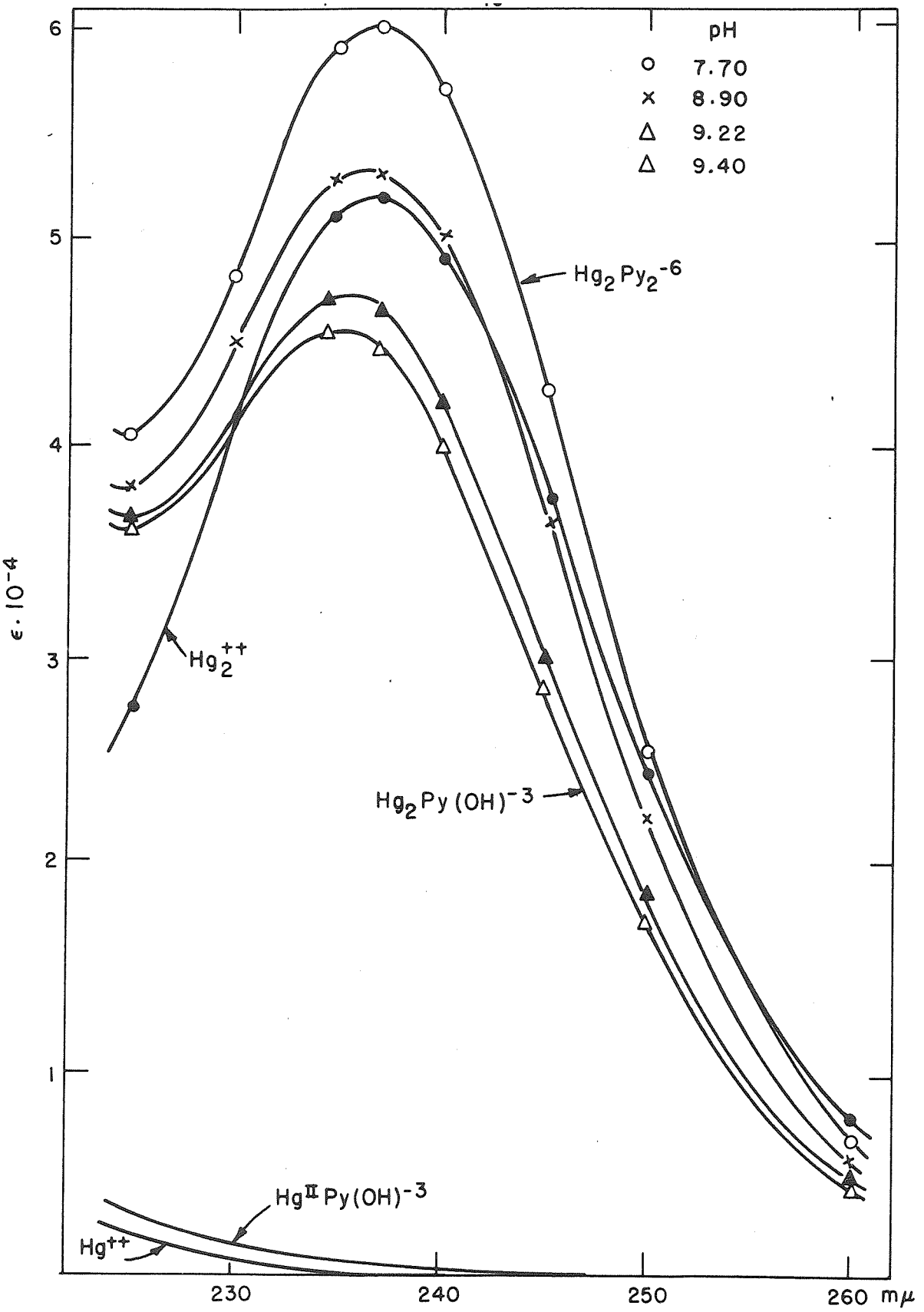

pH
7.70
8.90
9.22
9.40
$Hg_2Py_2^{-6}$
Hg_2^{++}
$Hg_2Py(OH)^{-3}$
$Hg^{II}Py(OH)^{-3}$
Hg^{++}
$\epsilon \cdot 10^{-4}$
6
5
4
3
2
1
230
240
250
260 mμ

Fig. 2

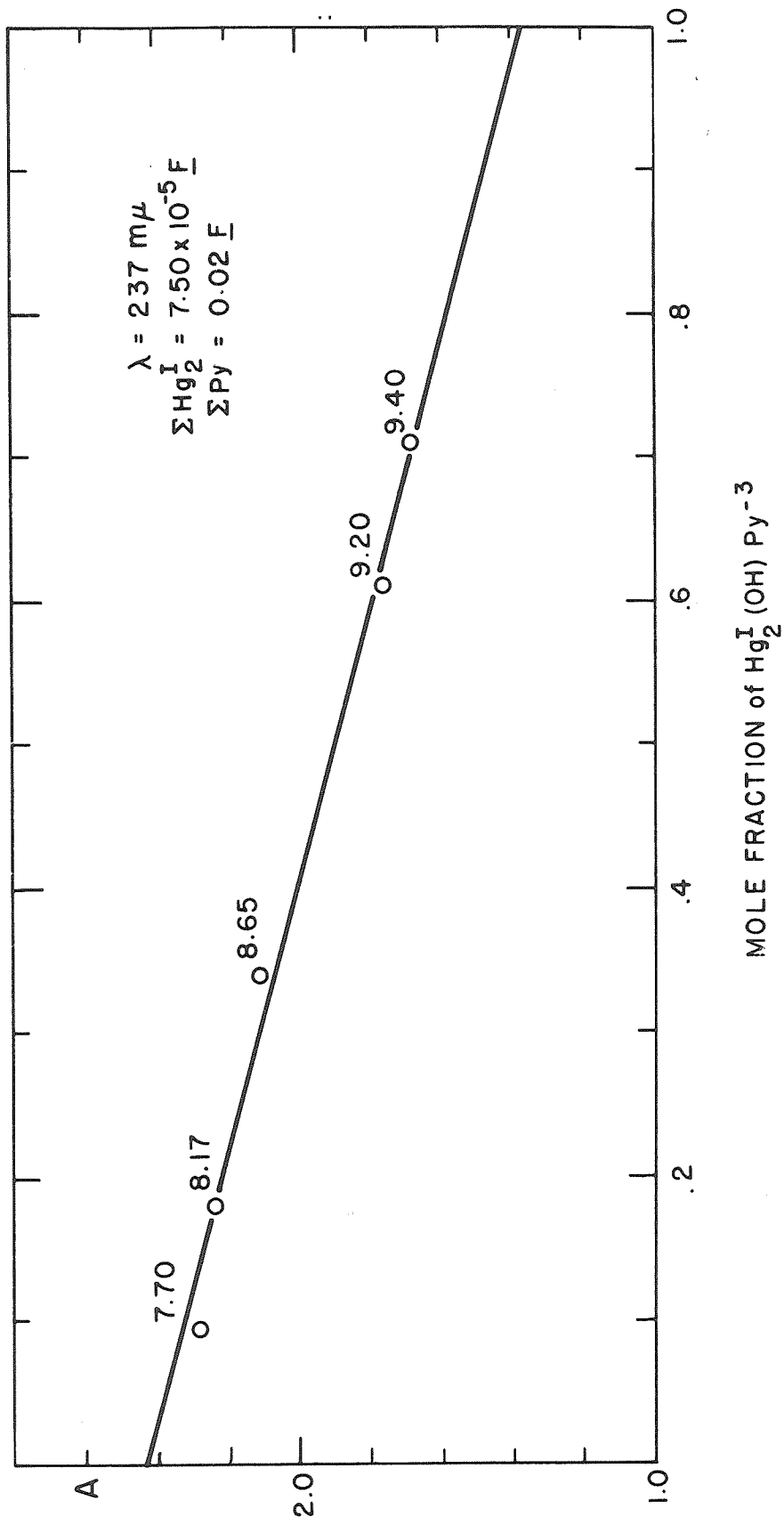

λ = 237 mμ
ΣHg$_2^I$ = 7.50 x 10^{-5} F
ΣPy = 0.02 F
7.70
8.17
8.65
9.20
9.40
A
2.0
1.0
.2
.4
.6
.8
1.0
MOLE FRACTION of Hg$_2^I$(OH) Py^{-3}

Fig. 3

the complexes $Hg_2^{I}Py_2^{-6}$ and $Hg^{II}(OH)Py^{-3}$. Roughly speaking, of the mercurous complexes, $Hg_2^{I}Py_2^{-6}$ is the main species at (ΣPy) = 0.02 F for pH's between 7 and 8.5; $Hg_2^{I}(OH)Py^{-3}$ is predominant in the pH range 9.3 to 10. In the strongly alkaline solutions, $Hg(OH)_2$ forms at low Py concentrations. There is no evidence for soluble complexes containing HPy^{-3} species; at pH's less than 6.5, however, insoluble pyrophosphate salts precipitate. A precipitate also forms in the pH range 7-9.5 if the Hg_2^{I} concentration is raised above 10^{-3}. (However, in 6 F $K_4P_2O_7$ solution, the Hg_2^{I} concentration could be raised to about 0.07 M before precipitation occurred).

Mercury (I) Complexes of Tripolyphosphate and Dicarboxylate Anions. - Fig. 4 shows the plots of equation 7 for the anions tripolyphosphate (Tp^{-5}), oxalate (Ox^{-2}), α-dimethylmalonate (Ma^{-2}) and succinate (Su^{-2}). The plots all indicate formation of $Hg_2^{I}L_2$ and $Hg_2^{I}(OH)L$ complexes. Especially for the dicarboxylic acids, the complexes are less stable than the pyrophosphate complexes, and rather high concentrations of ligand are needed to keep the potential below that for the formation of mercuric oxide. The values of K_1 and K_2 are given in Table V.

In all cases clear solutions were obtained on adding mercurous solution to the ligand solution, indicating no disproportionation, and in all cases calomel could be precipitated from these resulting clear solutions. The following quantita-

Fig. 4

Plots of equation 7 for the determination of $\underline{K}_1$ and $\underline{K}_2$; $(\Sigma Hg_2^I) \simeq 5x10^{-6} - 8x10^{-5}$ $\underline{F}$. Only a rather narrow range of Hg_2^{++} concentration could be used as Hg_2L precipitated at higher (Hg_2^I), and at lower (Hg_2^I) the emf's ceased to be well reproducible; $(\Sigma$ Tp$) \simeq 0.01 - 0.1$ $\underline{F}$; $(\Sigma$ Ox$) \simeq 0.05 - 0.7$ $\underline{F}$; $(\Sigma$ Ma$) \simeq 0.01 - 0.05$ $\underline{F}$; $(\Sigma$ Su$) \simeq 0.1 - 0.8$ $\underline{F}$; $\triangle$ refers to the potentials close to that of Hg,HgO electrode. The numbers next to the curves give the pH for the particular set of measurements.

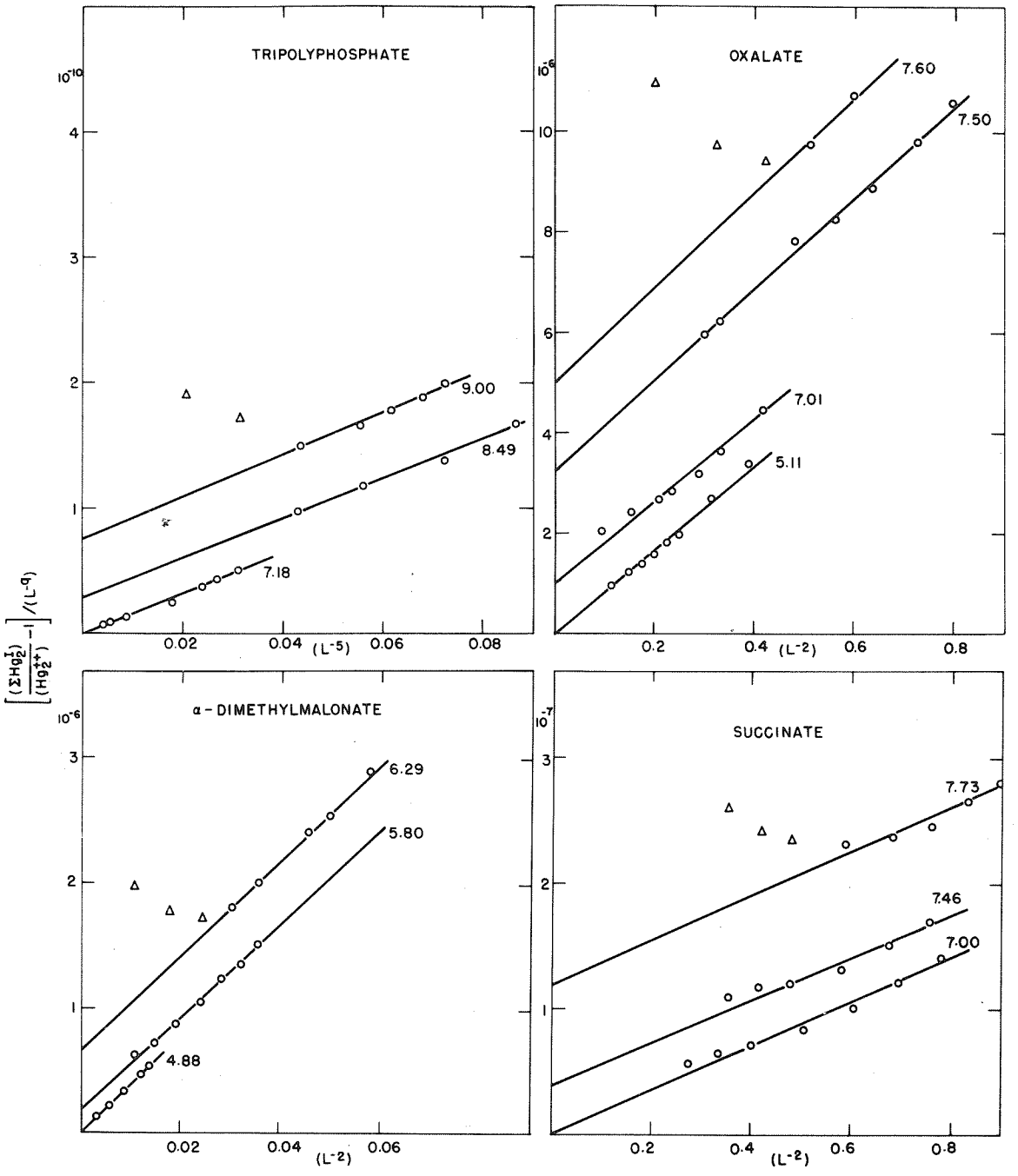
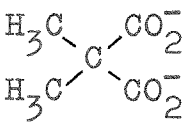
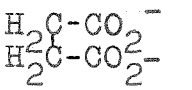

TRIPOLYPHOSPHATE
OXALATE
α-DIMETHYLMALONATE
SUCCINATE
9.00
8.49
7.18
7.60
7.50
7.01
5.11
6.29
5.80
4.88
7.73
7.46
7.00
(L⁻⁵)
(L⁻²)
[(ΣHgL)/(Hg²⁺) − 1]/(L⁻⁴)

Fig. 4

Table V

Complex Formation Constants

Ligand	$Hg_2L_2^{-2q+2}$ $\underline{K}_2$ $\underline{M}^{-2}$	$Hg_2(OH)L^{-q+1}$ $\underline{K}_1$ $\underline{M}^{-2}$
$P_2O_7^{-4}$	$(2.4\pm0.6)10^{12}$	$(4.4\pm0.6)10^{15}$
$P_3O_{10}^{-5}$	$(1.7\pm0.3)10^{11}$	$(1.0\pm0.2)10^{15}$
$C_2O_4^{--}$	$(9.5\pm0.2)10^{6}$	$(1.1\pm0.2)10^{13}$
	$(3.3\pm0.6)10^{7}$	$(3.8\pm0.5)10^{13}$
	$(1.9\pm0.3)10^{7}$	$(2.8\pm0.6)10^{13}$

tive experiment is of interest. A solution at pH 7.0, 0.30 $\underline{F}$ in sodium α-dimethylmalonate and containing $5.65\times10^{-4}\underline{F}$ Hg^{II} was stirred in the presence of elementary mercury for 15 hours. After separation from the mercury pool, the solution was made 1 $\underline{M}$ in $HClO_4$. It showed the characteristic Hg_2^{++} absorption spectrum. The concentration of Hg_2^{++} (by spectrophotometry and by precipitation of Hg_2Cl_2) and of Hg^{++} (dithizone extraction into $CHCl_3$) obtained were $(Hg_2^I) = 4.7\times10^{-4}\underline{F}$ and $(Hg^{II}) = 0.8\times10^{-4}\underline{F}$. According to Table V, the ratio of $Hg_2^I(OH)L$ to Hg_2L_2 should be about 3 in this solution. Thus the result clearly shows that the complexes obtained are Hg_2^I complexes, and that both $Hg_2Ma_2^{-2}$ and $Hg_2(OH)Ma^{-1}$ exist.

High concentrations of the complexing anions are also

necessary to prevent precipitation of insoluble salts.

It should be mentioned that non-reproducible results, potentials that varied with time, and the formation of a black precipitate were observed with unsubstituted malonic acid. There is probably mercuration of the reactive α-hydrogens and disproportionation to α-mercuric malonate and mercury. Malonic ester derivatives are known to form mercury derivatives.[15]

DISCUSSION

The values of $\underline{K}_1$ and $\underline{K}_2$ for all ligands studied are given in Table V. Tripolyphosphate has a negative charge of 5 and can be a tridentate ligand[16] and Hirschfelder-Taylor models indicate that bonding of the metal ion to the two terminal phosphate tetrahedra and to the center tetrahedron as well can occur without strain in either a square planar or an octahedral complex, whereas pyrophosphate has a charge of -4 and is bidentate. Tripolyphosphate is usually a much stronger complexer than is pyrophosphate, but this order is reversed for the mercurous complexes.

The dicarboxylate complexes are substantially weaker than the pyrophosphate complexes. The six-membered ring of α-dimethylmalonate appears to give slightly stronger complexing than for the 5 and 7 membered rings of oxalate and succinate respectively.

The square root of $\underline{K}_2$ may be taken as a rough estimate

for the binding of a single L^{-q} by Hg_2^{++}

$$Hg_2^{++} + L^{-q} = Hg_2L^{-q+2} \qquad \underline{K} \simeq \underline{K}_2^{1/2}$$

Then the ratio $\underline{K}_1/\underline{K}_2^{1/2}$ is an estimate of the equilibrium constant for the reaction, $Hg_2L^{-q+2} + OH^- = Hg_2(OH)L^{-q+1}$. It comes out fairly constant as can be seen in Table VI.

Table VI

Values of $\underline{K}_1/\underline{K}_2^{1/2}$

Ligand	$\underline{K}_1/\underline{K}_2^{1/2} \times 10^{-9}$ M
Pyrophosphate	2
Tripolyphosphate	3
Oxalate	4
α-Dimethylmalonate	6
Succinate	6
$Hg_2^{++} + OH^- = Hg_2OH^+$	$\underline{K} \simeq 1\times10^{9(12)}, 8\times10^{9(17)}$

Values given in the literature for the reaction

$$Hg_2^{++} + OH^- = Hg_2OH^+$$

are included in Table VI, and are of the same order of magnitude as $\underline{K}_1/\underline{K}_2^{1/2}$. Thus this very crude argument indicates that the binding of OH^- by Hg_2L^{-q+2} is approximately constant, independent of the nature of L and of the charge $-q+2$.

If L is a ligand which can form complexes with both

Hg^{I} and Hg^{II}, $Hg_2^{I}L_n$ and $Hg^{II}L_m$ respectively, we can write the following disproportionation equation:

$$Hg + Hg^{II}L_m = Hg_2^{I}L_n + (m-n)L.$$

What are the structural and electronic characteristics of L which make the mercurous complexes either stable or unstable for disproportionation?

We first recall that Hg^{++} tends to bind two monodentate ligands strongly in a linear configuration, and additional ligands are bound with much weaker affinity. In the crystal structure for many mercuric compounds, there are two short Hg-X distances in a linear configuration and additional long Hg-X bonds to give a distorted octahedral arrangement.[18] There are also some cases of tetrahedral HgX_4 structures, viz., crystalline HgI_2 and HgX_4^{--}[19] ($X = Cl^-, Br^-, I^-$) ions in solution, but HgI_2 and HgX_2[18] are linear in the gas phase. The first two binding constants for Hg^{++} with Cl^-, Br^- and I^- are much greater than $\underline{K}_3$ and $\underline{K}_4$.[20,21]

For the few crystal structures which are known for mercurous compounds, there are linear X-Hg-Hg-X structures, with additional ligands at larger distances around each Hg atom to give a distorted octahedral coordination. From an electronic and structural point of view, therefore, mercurous compounds are just like linear HgX_2 compounds, except that one of the bonds is a Hg-Hg bond.

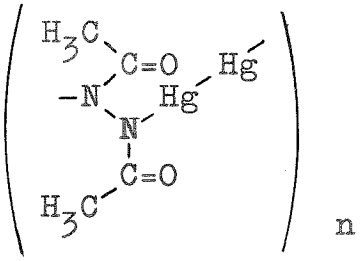
Table VII

Interatomic Distances in Some Mercurous Compounds

	Hg-Hg	Hg-X	Ref.
Hg_2F_2	2.43 Å	2.13 Å	22
Hg_2Cl_2	2.53	2.52	22
Hg_2Br_2	2.58	2.57	22
Hg_2I_2	2.69	2.68	22
$Hg_2N_2Ac_2$ *	2.90		23

* mercurous diacethydrazide

Table VII displays the mercury-mercury and mercury-ligand distances for some mercurous compounds. The substance mercurous diacethydrazide has the structure

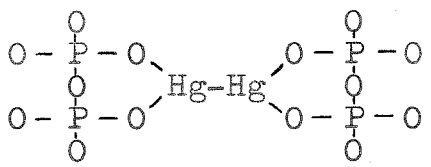


The binding constants for complex formation with Hg^{++} increase in the order, F^-, Cl^-, Br^-, I^- and NH_3[20,21, 24] and if we take a very rough point of view that the nitrogen in the diacetyl-hydrazine is about as good a complexer as is NH_3, then Table VII shows that the stronger the Hg-X bond, the longer and weaker the Hg-Hg bond.

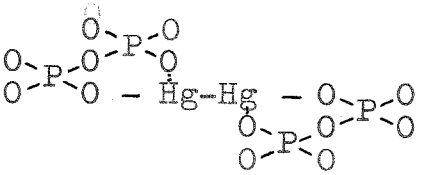
Ligands which form good covalent bonds to mercury such as NH_3 or CN^- cause disproportionation of the hypothetical

$Hg_2^{I}L_2$ into $Hg^{II}L_2$ complexes and elementary mercury. Thus, our hypothesis is that a tendency to form a strong covalent Hg-X complex weakens the Hg-Hg bond and leads to disproportionation. In order to get good mercurous complexes therefore, one should use strong "ionic" ligands, such as $P_2O_7^{-4}$, $C_2O_4^{--}$, etc., which complex by virtue of their charge and chelating characteristics. With these ligands, the contribution of covalent bond formation to the binding is small, as indicated by their relatively strong binding for the alkaline earth and group IIIb tripositive ions. These are in fact the complexing agents which do form mercurous complexes which are stable to disproportionation.*

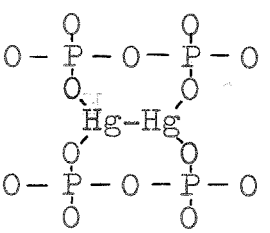
The structure of the mercurous pyrophosphate and other mercurous complexes which were studied here is an interesting problem in structural chemistry. The three structures,



A



B



C

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* Several interesting facts may be mentioned incidentally. R. Rosen and E.E. Reid report the existence of the compound

are all conceivable. The evidence that OH^- binds all $Hg_2^{I}L^{-q+2}$ ligands equally tends to indicate that the chelate L is attached to only one mercury as in structures A and B.

$HO-CH_2-CH_2-S-Hg-Hg-S-CH_2-CH_2-OH$, a yellow solid, soluble in hot alcohol and melting at 108°C.[25] It is not known whether this substance is stable to disproportionation. There are the curious facts that the substances $Hg_2(CO_2CX_3)_2$ (X = Cl or F) exist and are soluble in benzene.[26]

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THE INTERACTION OF $HgCl_2$ WITH DEOXYRIBONUCLEIC ACID

INTRODUCTION

An interesting reaction of calf thymus deoxyribonucleic acid (DNA) with $HgCl_2$ was first reported by Katz,[1] who found that the addition of $HgCl_2$ causes a drastic decrease in the viscosity of DNA solution, even at an ionic strength of 0.1 M, an effect not observed with any other salt. In the various studies on the effects of other inorganic salts on the intrinsic viscosity of solutions of DNA, the observed viscosity changes are much smaller, and in general most noticeable only in solutions with a low ionic strength.[2-5] Katz[1] further observed that the addition of halide or cyanide ions completely reverses the complexation and the initial viscosity is recovered. Besides this viscometric reversibility, Thomas[6] found that the ultraviolet absorption spectrum of DNA (λ_{max}= 258 mμ) is substantially altered by the addition of $HgCl_2$ (λ_{max} of DNA-Hg^{II} complex = 275 mμ) and could be reversed by the addition of sodium chloride. These spectral shifts are not observed when other cations are added.

In accordance with our interest in mercury complexes, the present study of the nature of the DNA-Hg^{II} complexes was undertaken.

EXPERIMENTAL

Calf thymus DNA, prepared by a modified Mirsky-Pollister procedure involving Sevagging and ethanol precipitation, was supplied by the Nutritional Biochemicals Corporation. These samples display an intrinsic viscosity of 73 dl/g, a protein content of ca. 1-2%, a molar absorptivity $\epsilon_{260}=6.65\times10^{3}$ cm^{-1} $(mole\ P)^{-1}$ and a phosphorus content of 7.3% bottle weight. From the studies of hyperchromicity both by heat and acid denaturation and also from the high viscosity displayed, we infer that the sample has a reasonably high molecular weight, i.e., it is a native sample.

M. lysodeikticus, D. pneumoniae and E. coli DNA were prepared by the Marmur procedure[8] and by a modified Avery procedure.[9] Pneumococcal DNA was prepared from a strain resistant to streptomycin, optochin and bryamycin. The pneumococcal DNA preparation was stored in a rather concentrated state (absorbance ca. 10) until use, in accordance with the widespread observation that dilute DNA solutions lose their biological activity.

Viscosity measurements were made with a viscometer following the design of Schneider,[10] with three bulbs and a 150 cm long capillary wound in a helix and led into an Ubbelohde-type mixing chamber. The capillary was 0.50 mm in radius. For this viscometer the three maximum shear gradients were ap-

proximately 220, 150 and 90 sec^{-1}. Concentration dependences were determined by running DNA solutions of about 10, 6 and 3 mg/dl.

All optical measurements were made with a Beckman Model DU spectrophotometer. A hydrogen lamp and quartz cells with a path length of 1.00 cm were used.

Sedimentation experiments were performed in a Spinco Model E analytical ultracentrifuge at a speed of 39,460 rpm. The sedimentation rate was measured in solution with a concentration of ca. 7 mg/dl, by an optical system based on light absorption. The values of the sedimentation coefficient, $S_{w,20}$, from absorption runs were determined from densitometer tracings. These sedimentation runs were kindly carried out by Mrs. J. Morris.

VISCOMETRIC EFFECTS

The interesting fact about the interaction of $HgCl_2$ with DNA (0.1 M in $NaClO_4$) is that even a small quantity of $HgCl_2$ is capable of lowering the intrinsic viscosity substantially. The loss in rigidity and viscous behavior of a rather concentrated DNA solution when mercury (II) is added can be visually observed and the rigidity is restored by addition of halide ions, cyanide ions, versene or any good complexer of mercury (II).

Figure 1 shows the effect of $HgCl_2$ on the intrinsic

viscosity of DNA solutions. A concentration range of r = 0 (r = total moles of added $HgCl_2$/ moles of P) to r = 2 in $HgCl_2$ was covered in the experiment. The DNA concentration was kept constant at 1.5×10^{-4} M in P. A relatively large initial drop in viscosity with r is observed which begins to level off at ca. r = 1, in accordance with Katz's finding.[1] It is important to note that the DNA solutions used here always contained 0.10 F $NaClO_4$ and 0.01 F sodium acetate (pH 5.7) before the $HgCl_2$ was added. The effect is the same even in 0.4 F $NaClO_4$ DNA solution. The effect of $Cu(ClO_4)_2$ is shown for comparison in Fig. 1. In this case, in order to produce a four-fold lowering in viscosity, an r of the order of 50 is required.[11] The effect of Mg^{++} is much smaller and the intrinsic viscosity of DNA is the same in 0.2 F $MgCl_2$ as it is in 0.2 F NaCl.[11] Iron (II) is also indicated to have a negligible effect.[12]

It is of interest that the addition of denaturing agents such as urea, guanidine hydrochloride and sodium salicylate does not affect the intrinsic viscosity of DNA solution at room temperature.[13,14] Formaldehyde in high concentrations (4 M) causes a decrease in the viscosity due to a slow reaction with the primary amino groups.[15]

Fig. 1

Effect of $HgCl_2$ on the intrinsic viscosity, $[\eta]$ in dl/g, of calf thymus DNA solution (1.5×10^{-4} in P), in 0.1 F $NaClO_4$ and 0.01 F sodium acetate, pH 5.7; r = moles of added $HgCl_2$/ moles of P. Effect of $Cu(ClO_4)_2$ under identical conditions is shown for comparison.

Fig. 2

Effect of $HgCl_2$ on the T_m of the calf thymus DNA. Viscosities measured at 25°C after 1 hr. exposure to the temperature noted on the abcissa. Measurements taken after removal of Hg(II) by the addition of NaCl.

Fig. 3

Effect of $HgCl_2$ on the rate of heat denaturation of DNA (calf thymus). Viscosity measured at 25°C after heating at a given temperature for a time interval noted on the abcissa. Conditions: 0.02 F $NaClO_4$, 0.005 F, pH 5.7, r=1.5. Viscosity measurements made after adding NaCl.

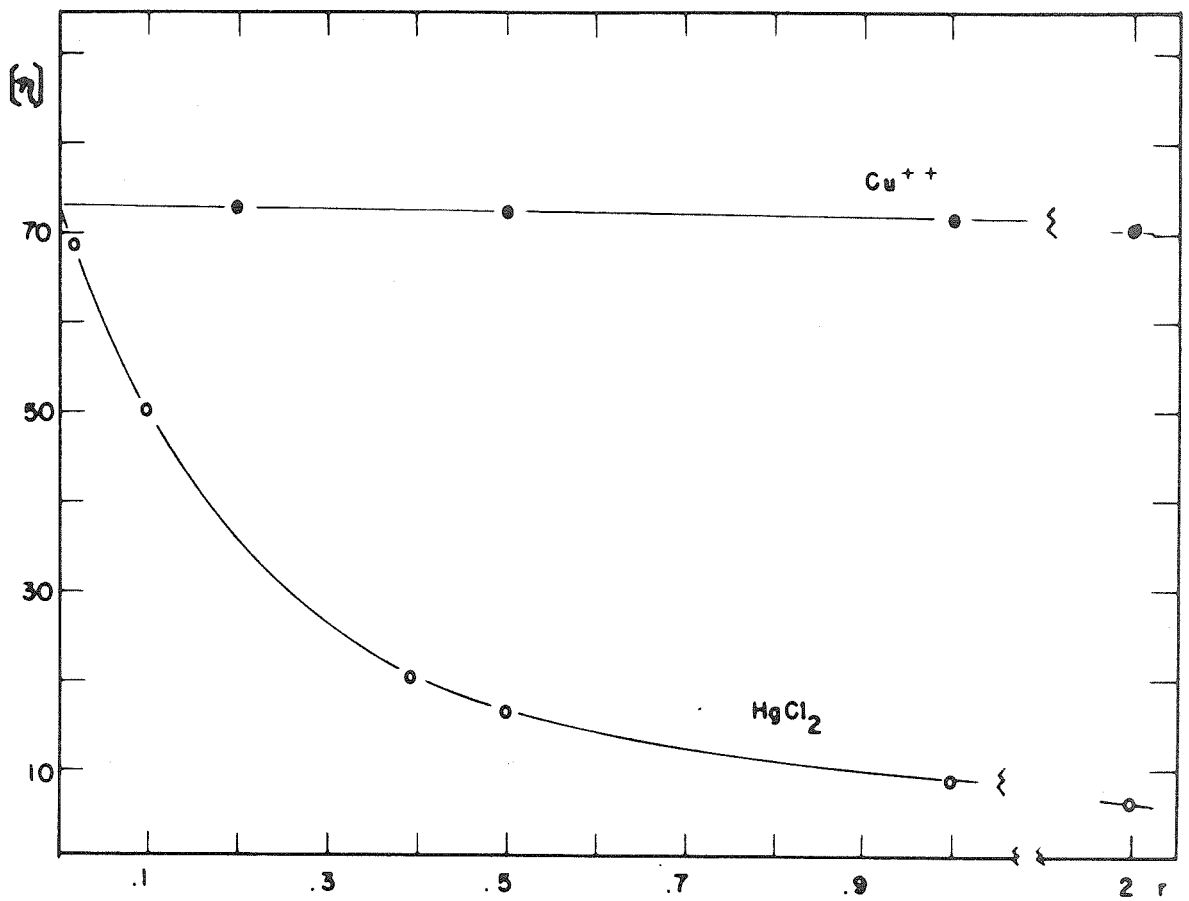

Fig. 1

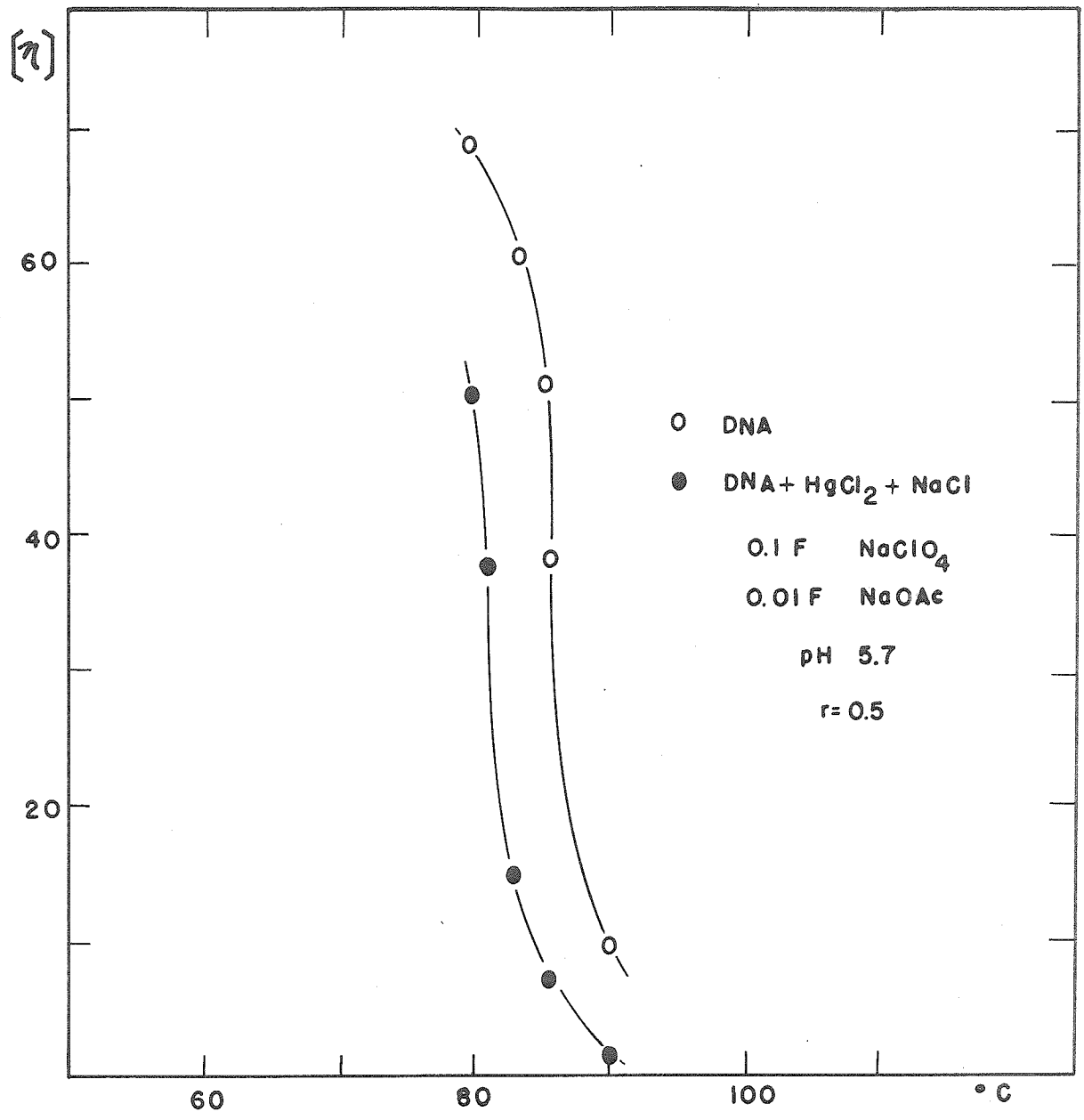
[η]
60
40
20
60
80
100
°C
O DNA
DNA + HgCl2 + NaCl
0.1 F NaClO4
0.01 F NaOAc
pH 5.7
r= 0.5

Fig. 2

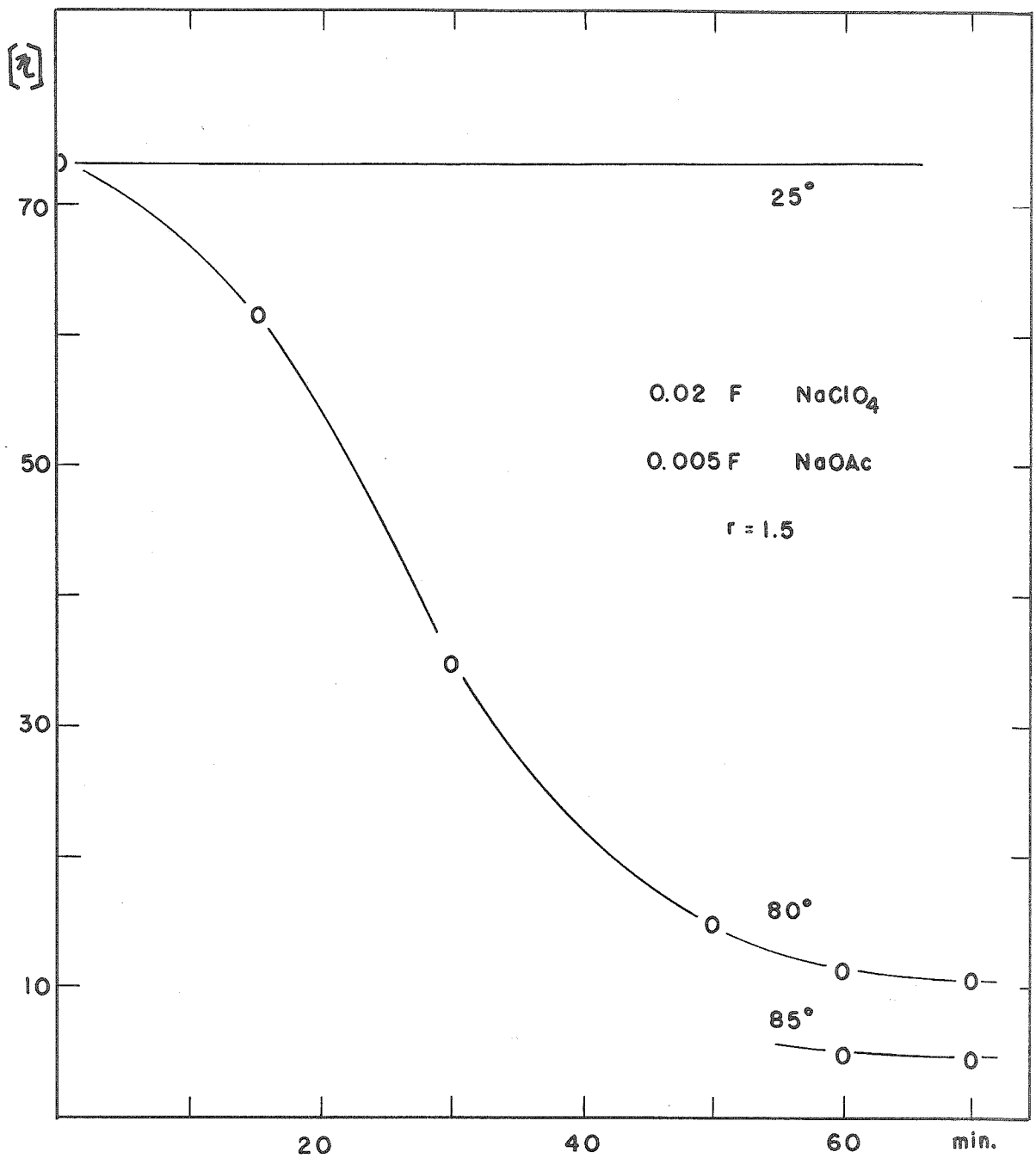

Fig. 3

HEAT DENATURATION EFFECT

The so called "melting temperature", T_m, is defined as the temperature at which 1 hour heating will reduce the intrinsic viscosity, measured at 25°C after rapid cooling, to one-half its original value.

Although ordinarily divalent cations affect T_m more strongly than monovalent cations, both effects are negligible at a level of 0.1 ionic strength ($NaClO_4$). Mercuric ion is the only known case which affects both T_m and viscosity very strongly at an ionic strength of 0.1 ($NaClO_4$) or higher. Measurements of the viscosity at 25°C, after rapid cooling, of a series of DNA solutions which had been heated at various temperatures in the presence of $HgCl_2$ for one hour were carried out. Results are shown in Fig. 2 for r=0.5. Measurements were made after removal of mercury (II) by the addition of NaCl. It is seen that the viscosity plot is shifted to lower temperatures by $HgCl_2$. The temperature at which the viscosity has fallen by half (T_m) is lowered ca. 5°, being ca. 7° for r=1. This order of lowering is smaller than in the case of 8 M urea (19°), 3.2 M guanidine hydrochloride (14°) and 0.8 M sodium salicylate (8°).[13,14] It should be mentioned that T_m is not affected by the presence of $HgCl_2$ in an excess of NaCl.

The nature of the denaturation process in the presence of $HgCl_2$ was examined by observing the change in viscosity as

a function of the time of exposure to a given temperature. The result is shown in Fig. 3, for r=1.5. Viscosity measurements were made at 25°C after adding NaCl. The main feature of this result is that the fall in viscosity levels out at a plateau which is lower the higher the temperature, indicating that the denaturation essentially comes to a stop short of completion by an amount dependent on the temperature.

SEDIMENTATION EXPERIMENT

The sedimentation experiments were carried out in 0.1 F $NaClO_4$, 0.01 F sodium acetate buffer (pH 5.7) with a concentration of 7 mg/dl for both undenatured and denatured DNA samples, and with r=0, 0.5 and 1.2. Results are displayed in Table I.

Table I

Sedimentation Coefficients of Mercurated DNA
(7 mg/dl)

	undenatured		denatured	
r	$s_{w,20}$	$[\eta]$	$s_{w,20}$	$[\eta]$
0.0	18.8	72	21.2	25
0.5	47.6	17	43.4	4
1.2	62.3	7	60.8	

It is evident from the Table I that we are not only dealing with an increase in the molecular weight due to the addition of the heavy ligand but also with a drastic change in

the shape or a collapse of the molecule.

Following the treatment recommended by Doty[16], molecular weights can be calculated by applying the Mandelkern-Flory[17] equation

$$M = \left| \frac{s^{o}[\eta]^{1/3}\,\eta_{o}\,N}{\beta(1 - \bar{v}\rho)} \right|^{3/2} \qquad (1)$$

where, s^{o}= the sedimentation constant at zero concentration, $[\eta]$= the intrinsic viscosity at zero gradient, η_{o}= the solvent viscosity, $N = 6.03\times10^{23}$, $(1 - \bar{v}\rho)$ = the buoyancy factor and β = a constant which is 2.6×10^{6} for a flexible coil and which for rigid coils depends on the molecular weight, increasing monotonically from 2.56×10^{6} at 300,000 to 3.29×10^{6} at 8×10^{6}.

The molecular weights obtained by using the values of $\beta = 3.29\times10^{6}$ for the undenatured DNA in the absence of mercury and $\beta = 2.6\times10^{6}$ for the denatured and the mercurated DNA, are given in Table II. Calculated molecular weights, assuming the complexing of Hg^{++}, are also shown in Table II.

Table II

Molecular Weights According to the Mandelkern-Flory Equation

r	undenatured	calc.	denatured	calc.
0.0	5.7×10^{6}		5.6×10^{6}	
0.5	9.8×10^{6}	7.7×10^{6}	4.2×10^{6}	7.4×10^{6}
1.2	7.6×10^{6}	10.3×10^{6}		

In the above calculation, the specific volumes of the

mercurated DNA's were obtained by using the equation

$$\bar{v}_{mixture} = \frac{w_1\bar{v}_1 + w_2\bar{v}_2}{w_1 + w_2}$$

where w_1 = the weight of DNA, $\bar{v}_1$ = the specific volume of DNA (0.55 ml/g),[18] w_2 = the weight of $HgCl_2$ added and $\bar{v}_2$ = the specific volume of $HgCl_2$ (0.0085 ml/g).[19]

All the above calculations are uncertain because (a) in the Mandelkern-Flory equation, s^o is the sedimentation constant at zero concentration, while in the present calculation $s_{w,20}$ of a DNA solution of 7 mg/dl was used, and (b) the use of $\beta = 2.6 \times 10^6$ for a partially denatured DNA is not quite valid. Due to the several factors involved, possibly aggregation, we cannot fully discuss the results obtained.

It is plausible to assume that Hg^{II} combines with the bases rather than with phosphate. This assumption is inferred from the general character of Hg^{II} complexes and corroborated by the spectral evidence which is discussed later. The viscosity decrease and the sedimentation constant increase may be due to a collapse or folding of the molecule without significant change in the degree of polymerization. It is conceivable, however, that there is an aggregation of the DNA molecules when Hg^{II} is added and this might be the cause or a part of the cause for the change in the physical properties.

If the bases combine with Hg^{II} and certain hydrogen bonds between the polynucleotide chains are destroyed, a more

flexible molecule would result which would tend to spontaneously assume a more condensed configuration. But since the original size and shape of the DNA molecule appears to be completely restored, after the removal of Hg^{II}, it is required that a sufficient number of pairings remain undestroyed in order to provide a "skeleton" to guide the reformation of the molecule when the reaction is reversed by the addition of any good complexer of Hg^{II}.

Katz[1] determined the molecular weight of the DNA-Hg^{II} complex by light scattering and interpreted the large molecular weight observed (16×10^6) as being the result of partial aggregation. However, he assumed that the complex had the same refractive index increment (dn/dc) as free DNA. A rough approximation of this correction can be made by assuming the additivity of refractive index increments, i.e., $(dn/dc)_{cmplx} = f_1(dn/dc)_{HgCl_2} + f_2(dn/dc)_{DNA}$, where f_1 = the weight fraction of Hg^{II}, f_2 = the weight fraction of DNA, $(dn/dc)_{HgCl_2} = 0.1$,[20] and $(dn/dc)_{DNA} = 0.19$.[21] This correction leads to a molecular weight not much different from that reported by Katz[1] and taken at face value, this result indicates dimerization of the double-strand DNA molecules when Hg^{II} is added.

In our opinion, the above result should be accepted with reservation, since the accuracy and the applicability of light scattering measurements, particularly for molecular

weights greater than 5×10^6, have been found to be unreliable.[16,22] On the other hand, nothing is known about the molecular form of the mercurated DNA so that the application of the Mandelkern-Flory equation based upon simple models is not justified. Probably its molecular weight cannot be satisfactorily settled until the sedimentation and viscosity data are rigorously interpreted.*

TRANSFORMATION EXPERIMENT

In order to determine whether the spectrophotometric and viscometric reversibility of the binding of $HgCl_2$ by DNA is also biologically reversible, biological titrations of mercurated pneumococcal DNA were carried out in collaboration with Mr. William F. Dove, Jr.

Two samples of active DNA were dissolved in 0.1 F $NaClO_4$, 0.01 F in sodium acetate and 5×10^{-3} F in $HgCl_2$. Ultraviolet spectra indicated that the mercurated samples had indeed been saturated (λ_{max}= 275 mμ). Both solutions were then dialyzed overnight, first against 0.01 F cysteine and 0.1 F NaCl and then against 0.1 F NaCl. After dialysis there was

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* Thomas[6] observed that the ethanolic precipitate of DNA in the presence of $HgCl_2$ is a fine powder which settles slowly. It is fibrous in the absence of $HgCl_2$.

no spectral evidence for the presence of mercuric ion.

Biological titrations of these two samples for both streptomycin and bryamycin resistant transforming factors gave results indicated in Fig. 4, which show that essentially no activity is lost by the cycle of mercuration and demercuration by dialysis, i.e., it remains "functionally intact". It is also known that 4 F urea does not cause inactivation of the transforming principle.[23]

Fig. 4 ⟶

Comparison of titration curves obtained with $HgCl_2$ treated and control samples of transforming DNA containing the streptomycin and bryamycin-resistance factors.

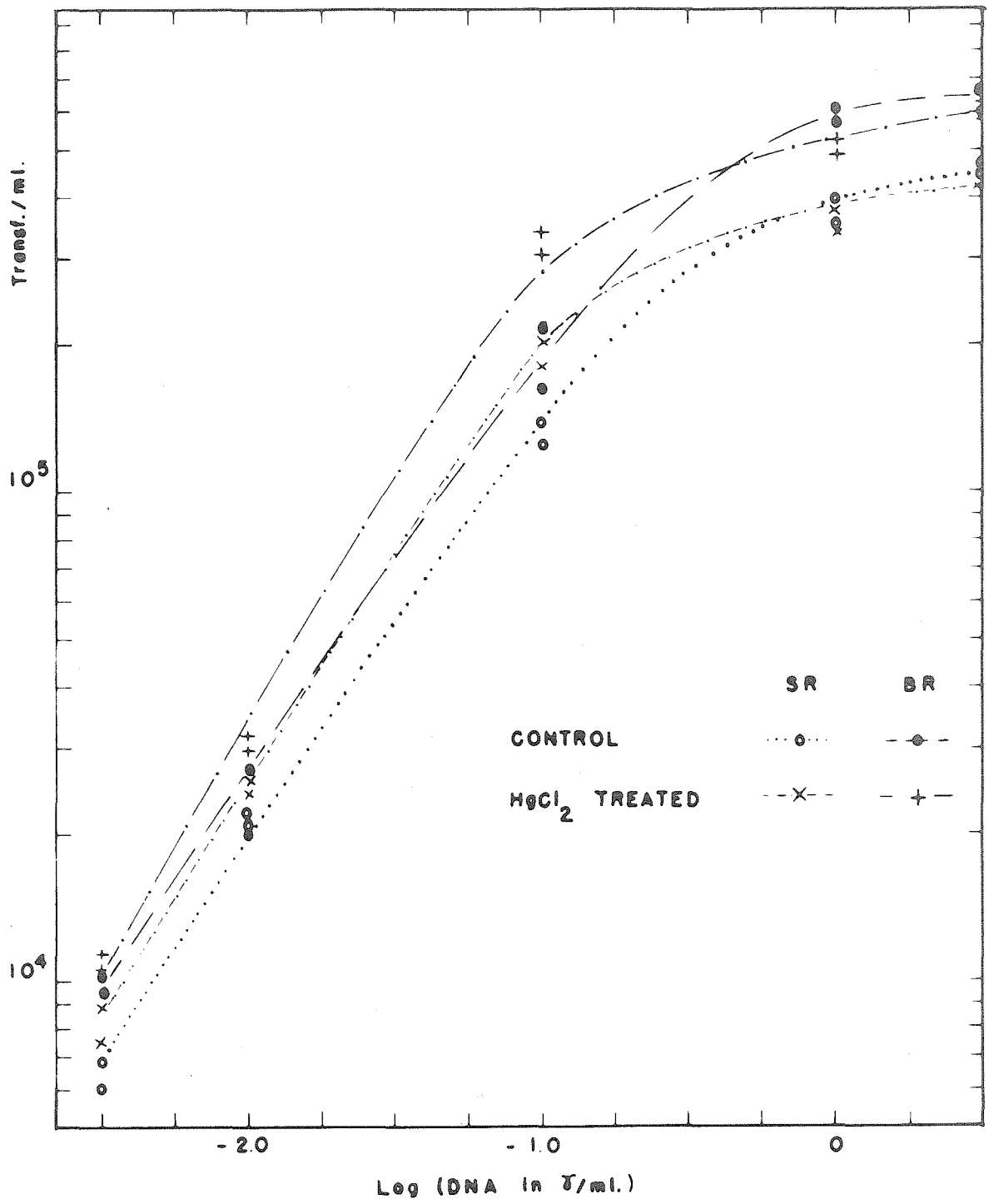

Transf./ml.
10^5
10^4
SR
BR
CONTROL
$HgCl_2$ TREATED
-2.0
-1.0
0
Log (DNA in γ/ml.)

Fig. 4

SPECTROPHOTOMETRIC AND TITRATION EXPERIMENTS BEARING ON THE NATURE OF THE COMPLEX

A. Mercury Complexes.

As background for the consideration of the nature of the DNA-Hg^{II} complex, a general discussion of the complex chemistry of Hg^{II} will be given here.

In general, for most inorganic cations, the consecutive complex formation constants, K_n, gradually decrease with increasing n, $K_1 > K_2 > \ldots > K_n$, which is partly due to purely statistical factors. The first ligand to be taken up would be bound somewhat more strongly than the subsequent ones. However, Hg^{II} presents $K_1 \simeq K_2$ and shows a drastic decrease in K_3 and K_4. This is due to the fact that Hg^{II} has a strong tendency to form two collinear sp bonds and resists a change in hybridization.

Table III

Formation Constants of Complex Ions[23]

	NH_3						Ethylenediamine		
	pK_1	pK_2	pK_3	pK_4	pK_5	pK_6	pK_1	pK_2	pK_3
Ni^{++}	2.80	2.24	1.73	1.19	0.75	0.03	7.66	6.40	4.55
Cu^{++}	4.15	3.50	2.89	2.13			10.72	9.31	-0.90
Cd^{++}	2.65	2.10	1.44	0.93			5.63	4.59	2.07
Hg^{++}	8.8	8.7	1.00	0.78			14.3	9.02	

	Imidazole	
	pK_1	pK_2
Cu^{++}	4.00	3.63
Cd^{++}	2.80	2.10
Hg^{++}	16.74	(β_2)[24]

Table III shows the binding constants of several nitrogen bases for various metal ions and several significant trends are evident in it. In the first place, there is a gradual decrease in K_1 to K_6 for Ni^{++} (coordination number 6), K_1 to K_4 for Cu^{++} (coordination number 4), etc., but in the case of Hg^{++} K_1 and K_2 are approximately equal and K_3 and K_4 are very small. The binding constants for imidazole and ammonia for complexing metals are of the same order of magnitude. The increase in binding from ammonia to ethylenediamine,(en), is very marked for all of the metals except Hg^{++}. Since ethylenediamine is a bidentate ligand, chelate formation undoubtedly contributes very significantly to the binding for the other metals. Because of the tendency to form two strong linear sp bonds, chelate formation contributes only slightly to the ligand binding for Hg^{++}. In fact the ratios, $pK_1(en)/p\beta_2(NH_3)$, where $\beta_2=K_1K_2$, are 1.52 (Ni^{++}), 1.40 (Cu^{++}), 1.19 (Cd^{++}) and 0.82 for Hg^{++}. Hg^{++} forms the strongest complexes with nitrogen bases of any of the cations.

Another interesting example is the hydrolysis of Hg^{II} ion. In the sequence of reactions

$$Hg(H_2O)^{++} = Hg(H_2O)OH^{+} + H^{+} = Hg(OH)_2 + 2H^{+}$$

with $pK_1 = -3.70$ and $pK_2 = -2.60$,[23] the second stage proceeds more easily than the first, and the fraction of the total Hg^{II} content present in the form of $Hg(OH)^{+}$ will never exceed about

12%. At pH > 4, Hg^{II} is present entirely as $Hg(OH)_2$.

In general the order of thermodynamic stability of the halide complexes of metal cations in aqueous solution is $F^- > Cl^- > Br^- > I^-$, which is expected from an electrostatic point of view. However, Hg^{II} belongs to a small group of metal ions, Cu^I, Ag^I, Cd^{II} and Pt^{II} which form halogen complexes with the opposite sequence of stabilities, viz., $I^- > Br^- > Cl^- > F^-$ (Table IV). For these metal ions something other than purely electrostatic forces must contribute to the metal-halogen bond strengths. Chatt[25] points out that for the above metal ions

Table IV

Mercury Halide Complexes[23]

	Cl^-	Br^-	I^-
pK_1	6.74	8.94	12.87
pK_2	6.48	7.94	10.95
pK_3	0.85	2.27	3.78
pK_4	1.00	1.75	2.23

the greater stability of the chloro complexes relative to the fluoro has its analogy in the much greater stability of thioether (R_2S) complexes relative to ether (R_2O) complexes and also trialkylphosphine (R_3P) complexes relative to trialkylamine (R_3N) complexes. Since the ligand atoms F, O and N have no vacant orbitals, whereas Cl, S and P have vacant d orbitals, it is suggested that the greater metal-ligand bond strength is a result of π bonding.[25] In the case of iodide,

the ion-induced dipole binding is likely to be an important factor due to the high polarizability of this ion.

Provided steric effects do not interfere, the above mentioned metal ions show coordinating affinities in the sequence $N \gg O \gg F$. It is of interest that it is just the ions showing the increased stability of iodide over fluoride complexes in solution that are known to form olefin complexes.

B. Complexes A and B.

We now consider the spectral study of the DNA-Hg^{II} complex.

Thomas[6] observed that for the calf thymus DNA, all curves for which $r < 0.6$ pass through an isosbestic point at 262.5 mμ. On increasing the $HgCl_2$ concentration from $r = 0.6$ to 10, the absorption curves display a new isosbestic point at 274.5 mμ and he concluded that the reaction proceeds by at least two steps and that the first reaction is essentially complete before the second one begins.

We have confirmed his observations (Fig. 6) except for the isosbestic point at 238.5 mμ which he found for all r. This discrepancy may be due to different samples of DNA. It should be mentioned that Beer's law holds for solutions with r up to 0.5 and this is indicative of the presence of one type of complex. Identical spectra are obtained either with $Hg(ClO_4)_2$ or $HgCl_2$.

Fig. 5

Plot of A(absorbance) = $\log_{10}(I_o/I)$ of some purine and pyrimidine bases and adenosine in the presence of $Hg(ClO_4)_2$. r = moles of $Hg(ClO_4)_2$/moles of base. Conditions: 0.10 F $NaClO_4$ for 5a, b, c and d; 0.4 F sodium acetate (pH 5.7) for 5e and f. For 5e, r = moles of $HgCl_2$/ moles of adenine.

Fig. 6

Effect of $HgCl_2$ on the ultraviolet absorption spectra of DNA of calf thymus (42% G-C), E. coli (50% G-C) and M. lysodeikticus (72% G-C). Spectra taken in 0.10 F $NaClO_4$, 0.01 F sodium acetate buffer, pH 5.7, with increasing r; r = moles of $HgCl_2$/moles of P.

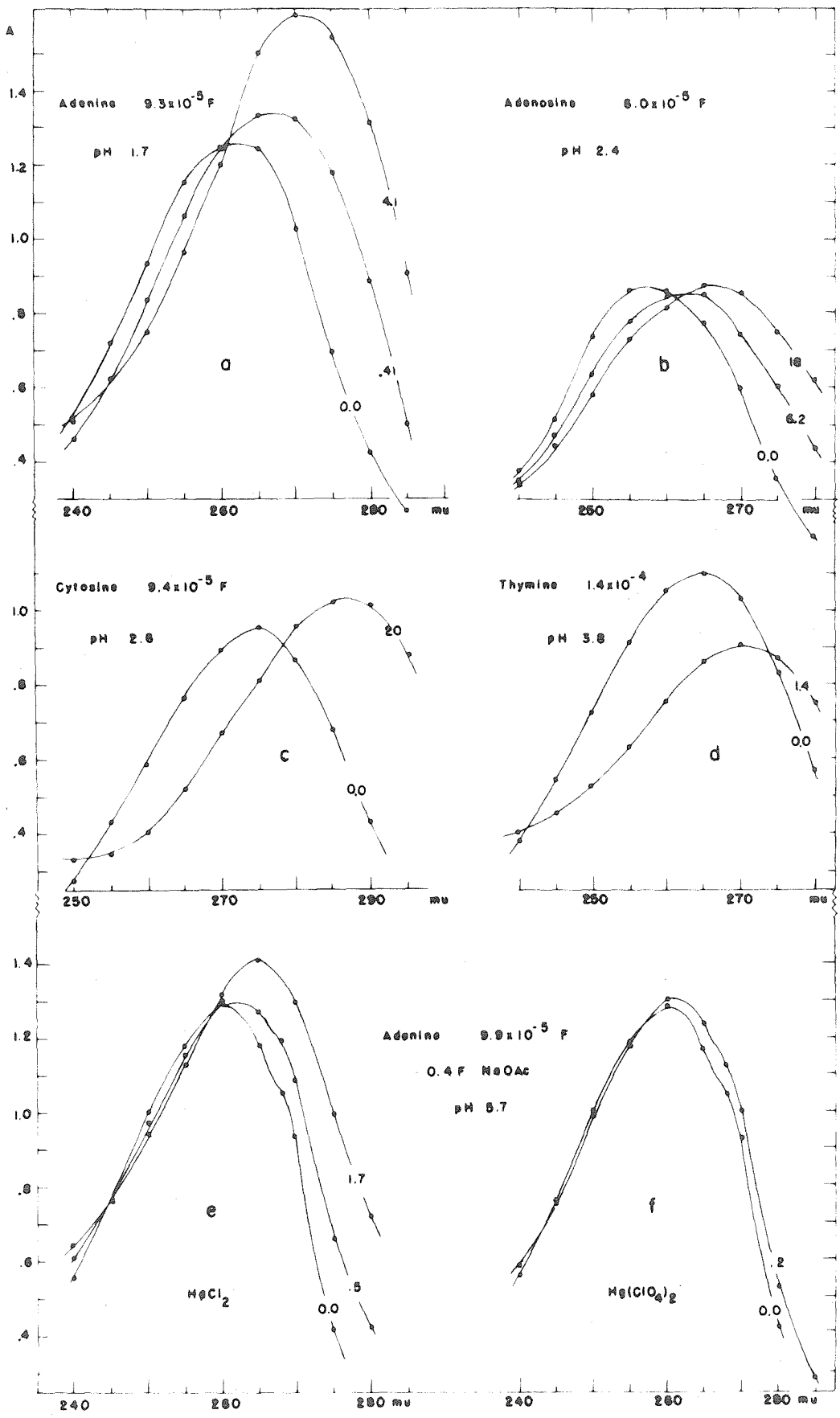

A
Adenine 9.3x10⁻⁵ F
pH 1.7
4.1
.41
0.0
a
240
260
280 mμ
Adenosine 8.0x10⁻⁵ F
pH 2.4
18
6.2
0.0
b
250
270 mμ
Cytosine 9.4x10⁻⁵ F
pH 2.8
20
0.0
c
250
270
290 mμ
Thymine 1.4x10⁻⁴
pH 3.8
1.4
0.0
d
250
270 mμ
Adenine 9.9x10⁻⁵ F
0.4 F NaOAc
pH 5.7
1.7
.5
0.0
e
HgCl2
.2
0.0
f
Hg(ClO4)2
240
260
280 mμ

Fig. 5

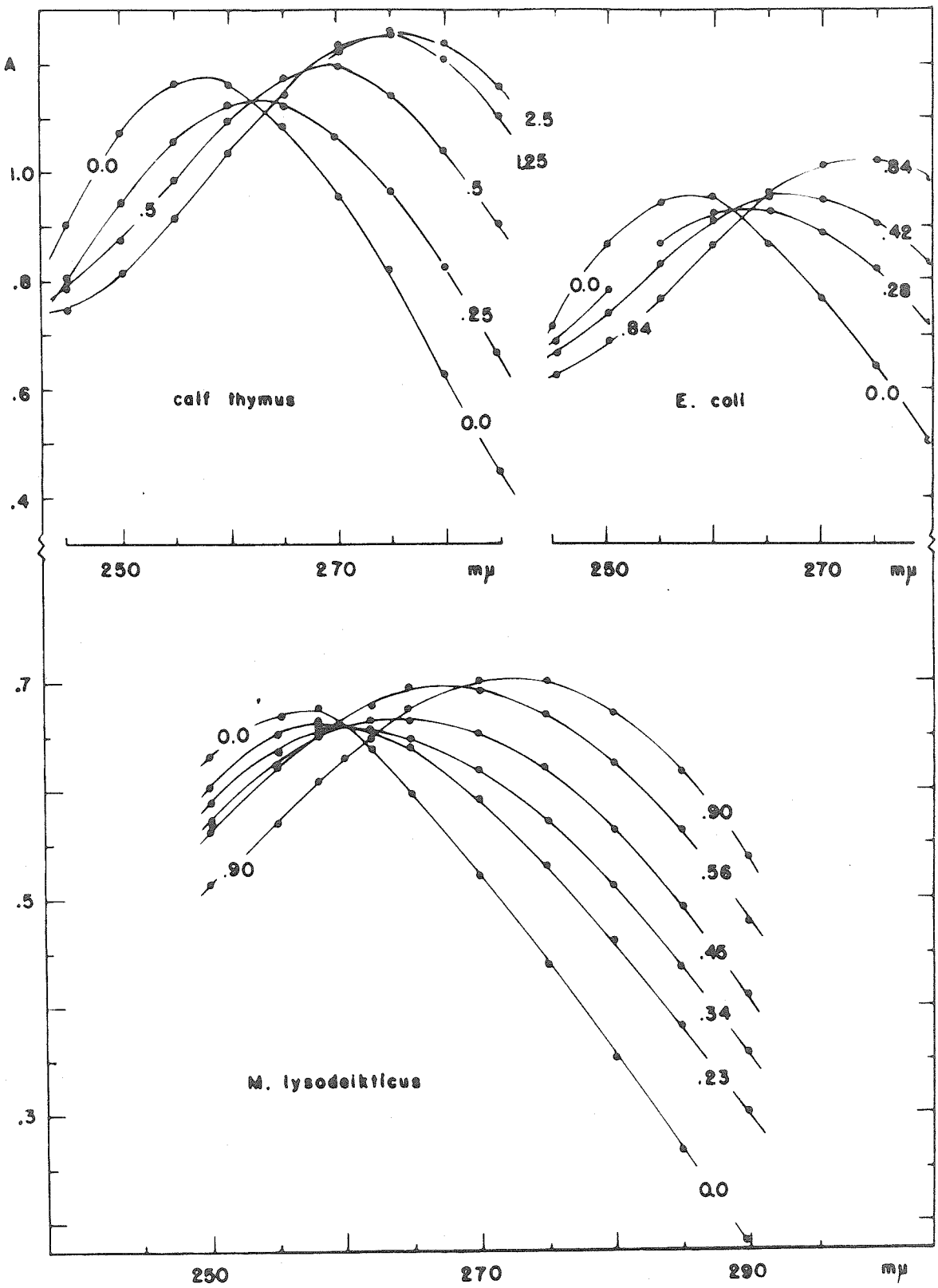

A
1.0
.8
.6
.4
0.0
.5
2.5
1.25
.5
.25
0.0
calf thymus
250
270
mμ
.84
.42
.28
0.0
.84
0.0
E. coli
250
270
mμ
.7
.5
.3
0.0
.90
.90
.56
.46
.34
.23
0.0
M. lysodeikticus
250
270
290
mμ

Fig. 6

For simplicity let the first complex be called A and the second complex B.

It is a well established fact that the purine and pyrimidine bases of DNA are primarily responsible for the absorption maximum at 260 mμ. The change in the absorption curve and the shift in λ_{max} caused by the addition of Hg^{II} strongly indicates that the binding is taking place at these bases. The spectra of Hg^{II} with some nucleosides and bases are shown in Fig. 5 and the similarity of these spectra to that of the DNA-$HgCl_2$ strongly suggests that Hg^{II} is bound to bases in DNA. The spectrum of the adenine-$HgCl_2$ system in 0.4 F sodium acetate, pH 5.7, is shown in Fig. 5e and no appreciable difference exists between this and that taken with $Hg(ClO_4)_2$ under identical conditions (Fig. 5f).

The fact that complex A is formed up to $r \simeq 0.5$, i.e., one mercury bound per one nucleotide pair, leads to one of the two following possibilities: (a) the base pair adenine-thymine or guanine-cytosine complexes with two atoms of mercury concomitantly, or (b) one atom of mercury binds to an adenine-thymine and another to a guanine-cytosine pair. In the present discussion we neglect the possibility of the aggregation of DNA molecules.

If the first hypothesis is the correct one, the saturation of the complex A should depend on the base composition,

i.e., if a given DNA presents 10% adenine-thymine or guanine-cytosine content, then the complex A should be formed up to r = 0.1. In order to elucidate this point, DNA from M. lysodeikticus (72% G-C) was prepared and spectra of the mercury complex were taken. As shown in Fig. 6, the spectra are almost identical to those of the calf thymus (42% G-C) and E. coli (50% G-C) DNA's, showing an isosbestic point at 261 ± 1 mμ for r up to 0.5. Therefore we can conclude that the formation of the complex A is entirely independent of the base composition and one should expect an isosbestic point at 261 ± 1 mμ up to r = 0.5 for any kind of DNA, and this indicates that the complex A is probably due to the binding of one mercury to an adenine-thymine pair and another to a guanine-cytosine pair.

The Hg^{II} spectrum is not much altered in a phosphate solution. Although the strength of the Hg^{II}- phosphate complex is not known, we contend that it is much weaker than those of the Hg^{II}- nucleosides.

C. Chloride Dependence.

The next question to be asked is whether $HgCl_2$ is bound to DNA as Hg^{++}, as $HgCl^+$, or in some other form.

Since up to r = 0.5 an average of one Hg^{II} is bound to one nucleotide pair, the complex formation reaction can be written as (charges are not balanced)

$$(\text{base-pair}) + HgCl_2 = ((\text{base-pair})\cdot HgCl_m) + (2-m)Cl^- \quad (1)$$

where (base-pair) = moles of P/2, and the constant of dissociation, K, is given by the equation

$$\frac{(\text{base-pair})(HgCl_2)}{((\text{base-pair})\cdot HgCl_m)} = K\,(Cl^-)^{2-m} \qquad (2)$$

In the above equations, (2-m) is the number of chloride ions displaced from $HgCl_2$ and corresponds to the slope of the plot of log $\{(\text{base-pair})(HgCl_2)/((\text{base-pair})\cdot HgCl_m)\}$ versus log (Cl^-).

In order to study the chloride dependence, various spectra were taken after adding a known concentration of chloride to the solutions with r<0.5 and 0.10 F in $NaClO_4$ and 0.01 F in sodium acetate (pH 5.7). Necessary corrections were made for the chloride liberated from $HgCl_2$. It should be mentioned that this analysis is valid only in the range of the chloride concentration where $HgCl_2$ is the predominant species. The relative concentrations of $HgCl^+$, $HgCl_2$, $Hg(OH)^+$ and $Hg(OH)_2$ at pH 5.7 are given in Table V, which indicates that under our experimental conditions, $HgCl_2$ is the predominant species in the absence of DNA. Since the experiments were done in 0.01 F sodium acetate, in calculating the relative concentrations of Hg^{II} chloride and hydroxo complexes, we have considered that the Hg^{II}- acetate complex is not important.

The result of this analysis is shown in Fig. 7, and a slope = 2 was obtained, which indicates that $HgCl_2$ loses both

Table V

Relative Concentrations of Hg^{II} Chloride and Hydroxo Complexes (pH 5.7)

Cl^-	$\frac{HgCl^+}{Hg^{++}}$	$\frac{HgCl_2}{HgCl^+}$	$\frac{HgCl_3^-}{HgCl_2}$	$\frac{HgCl^-}{HgOH^+}$	$\frac{HgCl_2}{Hg(OH)_2}$
10^{-5}	55	31	7×10^{-5}	0.55	0.013
10^{-4}	550	310	7×10^{-4}	5.5	1.32
10^{-3}	5500	3100	7×10^{-3}	55	132

chloride ions when it complexes with DNA.

Fig. 7 ⟶

Plot of equation 2 for the determination of (2-m) = slope. Conditions: $r<0.5$, 0.01 F sodium acetate (pH 5.7) and 0.10 F $NaClO_4$.

Fig. 8 ⟶

Plot of pH-stat (5.43) titration. The $Hg(ClO_4)_2$ solution used was 5.0×10^{-3} F in Hg^{II} and 5.0×10^{-3} F in $HClO_4$.

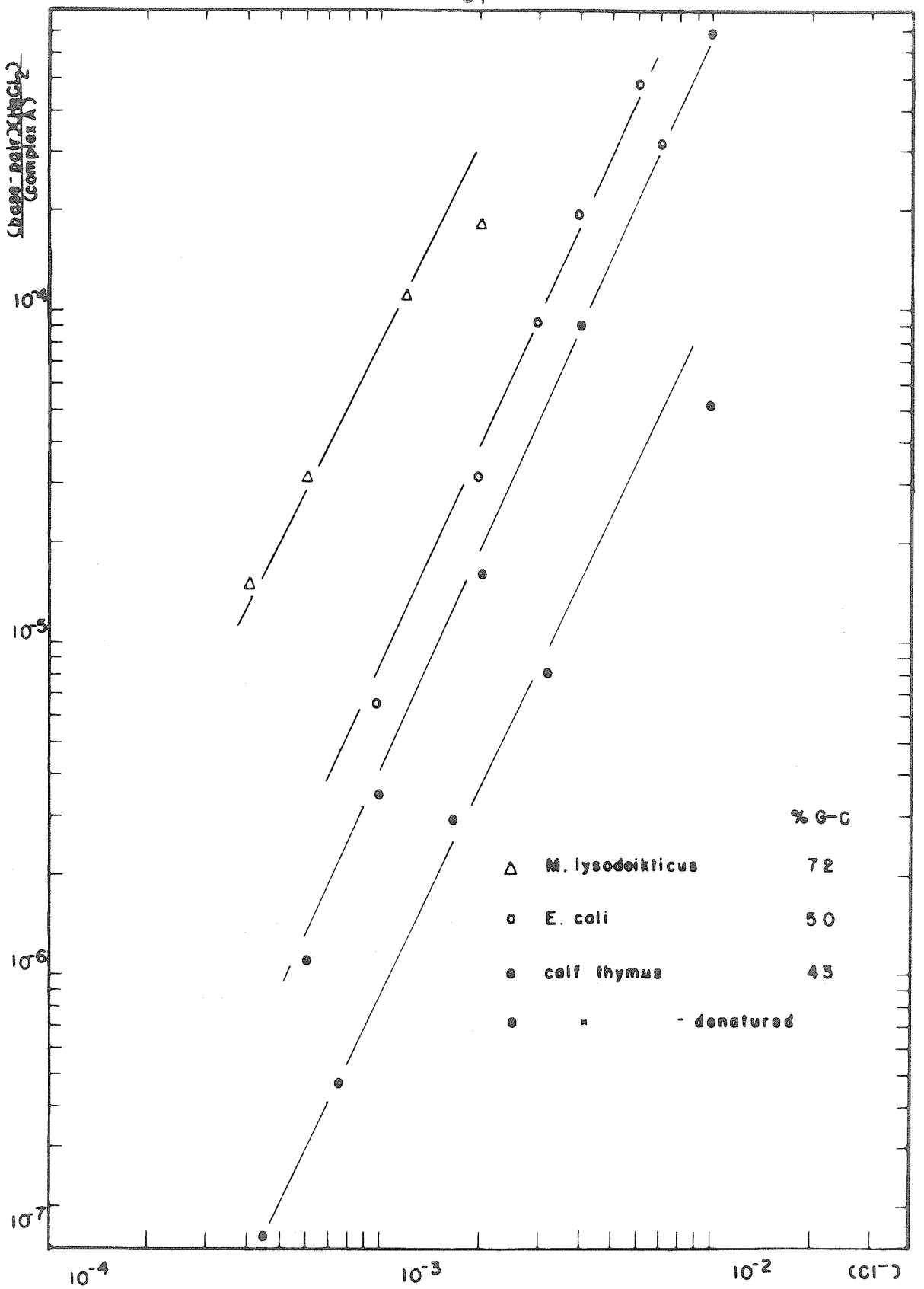

(base-pair $MgCl_2$ complex)$^{-1}$
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-4}
10^{-3}
10^{-2}
(Cl^-)
% G-C
M. lysodeikticus 72
E. coli 50
calf thymus 43
- denatured

Fig. 7

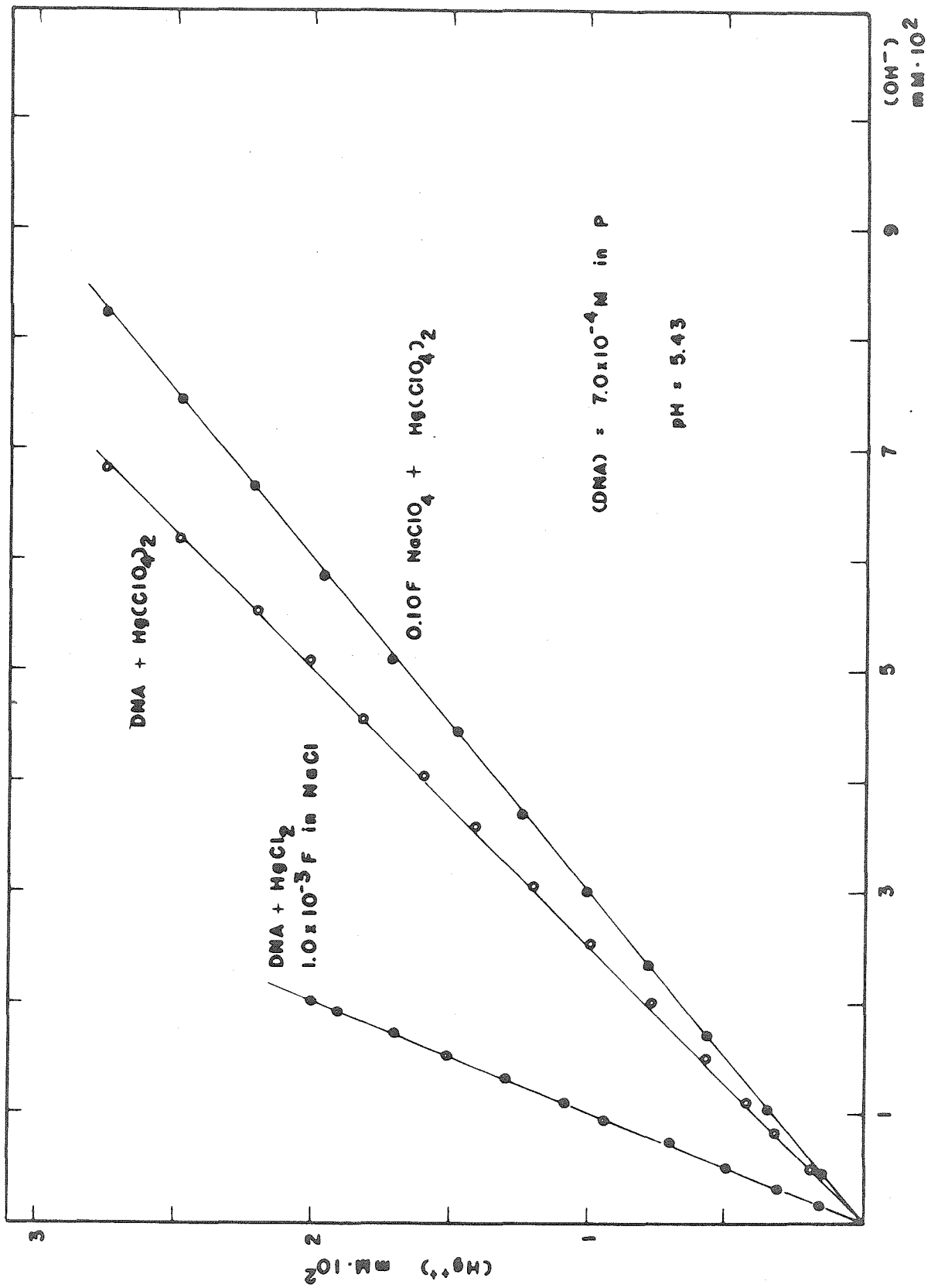

DMA + $Mg(ClO_4)_2$
DMA + $MgCl_2$
1.0×10^{-3} F in NaCl
0.10 F $NaClO_4$ + $Mg(ClO_4)_2$
(DNA) = 7.0×10^{-4} M in P
pH = 5.43
(H_{ext}^{+}) mM·10^2
(OH^{-}) mM·10^2
1
2
3
5
7
9

Fig. 8

D. pH Dependence.

The pH dependence on the binding of Hg^{II} to DNA was studied by carrying out a pH-stat titration by adding $Hg(ClO_4)_2$ to a DNA solution and neutralizing the acid liberated to the initial pH. The blank titration, i.e., the addition of $Hg(ClO_4)_2$ solution (5.00×10^{-3} F in Hg^{II} and 5.00×10^{-3} F in $HClO_4$) to 0.10 F $NaClO_4$ solution with the pH initially adjusted to 5.43, indicated, when corrected for the free protons, the liberation of 2 millimoles of protons per millimole of Hg^{II} added(Fig. 8). These two protons result from the hydrolysis of the Hg^{++} ion

$$Hg^{++} + 2HOH = Hg(OH)_2 + 2H^+$$

The same pH-stat titration carried out on adding Hg^{II} to DNA (7.0×10^{-4} M in P), in the absence of chloride, gave 1.5 millimoles of protons per millimole of Hg^{II} added (Fig. 8). This result can be interpreted in three ways: (a) we can assume that one Hg^{II} liberates one H^+ from DNA and binds to it in the form of base-Hg-base and another Hg^{II} also displaces one H^+ from DNA but undergoes hydrolysis, binding to DNA as base-HgOH; (b) an alternative interpretation is to assume that one Hg^{II} displaces $2H^+$ and another none but this one undergoes hydrolysis, and (c) two Hg^{II} displace $3H^+$ from the DNA.

A pH-stat titration carried out in the presence of 1.0×10^{-3} F NaCl, indicated liberation of one H^+ per Hg^{II} added

(Fig. 8). In this range of chloride ion concentration, (a) $HgCl_2$ is predominant rather than $Hg(OH)_2$ and the hydrolysis of Hg^{II} can be completely neglected (see Table V), and (b) the chloride concentration is not sufficiently high to reverse the equilibrium of the DNA-Hg^{II} complex.

Therefore, from the pH-stat titration experiments, it is conclusive that when the chloride concentration is low, one Hg^{II} undergoes hydrolysis and binds to DNA as base-HgOH, while another Hg^{II} attaches to DNA as base-Hg-base.

The above result is in contradiction with that obtained spectrophotometrically, because if Hg^{II} can bind to DNA in two different ways, the equation (1) takes the form

$$2(\text{base-pair}) + 2HgCl_2 = (\text{base-pair})Hg^{II} + (\text{base-pair})HgCl + 3Cl$$

where $((\text{base-pair})Hg^{II}) = ((\text{base-pair})HgCl)$. Therefore, from the plot of $\log \left[(\text{base-pair})^2(HgCl_2)^2/(\text{complex A})^2\right]^{1/2}$ vs. $\log (Cl^-)$ we should obtain a slope of 1.5 instead of 2 because at $(Cl^-) > 10^{-3}$ F and pH 5.7 there is no possibility of hydrolysis. Since the spectrophotometric experiments were carried out in 0.01 F sodium acetate, it is possible that acetate might have replaced chloride, base-Hg-Cl → base-Hg-OAc. Mercuric acetate salt in aqueous solution is known to be only slightly dissociated.[26]

From our general experience we infer that the spectrophotometric result is somewhat unreliable. The mass action law is not always obeyed too accurately. It is possible that

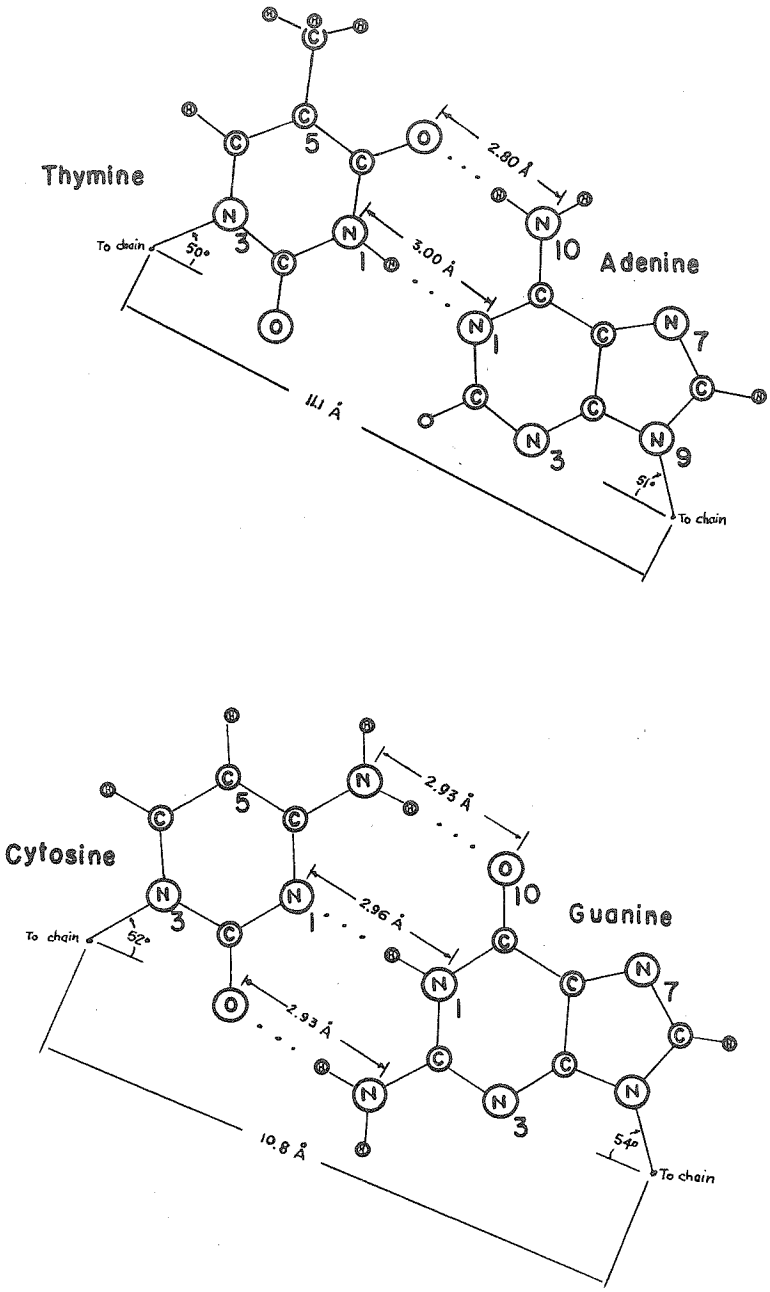
the discrepancy noted above is due to experimental error; it is also conceivable that it is due to the formation of a base-Hg-OAc complex. However, we are rather inclined to rely on the pH-stat titration results, and to assume that there are two types of Hg^{II}, one which loses two chlorides and binds to DNA as base-Hg-base and another which attaches as base-Hg-Cl or base-Hg-OH. It seems plausible to assume that protons are liberated from the bases and not from phosphates since it is generally believed that phosphates in DNA are not protonated at pH 5.4.[27]

E. Binding Sites.

Since the mercury (II) binding by the bases, nucleotides and adenine-thymine and guanine-cytosine polymers is not known, we can only speculate on the probable locus or loci of binding of the Hg^{II} to DNA.

According to molecular orbital calculations,[28] N_1 is the most basic nitrogen on the skeleton of purine, and N_7 in guanine. It is also confirmed by the X-ray study of the crystals of adenine hydrochloride that the proton is attached to N_1 in adenine.[29]

In the case of Complex A, it is conceivable that one mercury is bound to an adenine-thymine pair, probably between adenine N_1 and thymine N_1, displacing the hydrogen of the thymine N_1 (Fig. 9). The adenine-N_1 - thymine-N_1 distance is



Reproduced from L. Pauling and R. B. Corey, Archiv. Biochem. Biophys., 65, 164-181 (1956)

Fig. 9

3.00 Å and since a Hg^{II} ion requires about 4 Å, this can cause an opening up of the structure and may lead to a collapse of the molecule. Then from the pH-stat titration result, another Hg^{II} should also liberate one H^+ from DNA and coordinate either with OH^- or Cl^-. We suppose that the second one is going to a peripheral position, to either adenine N_7 or guanine N_7. However, it is difficult to see how this one can displace one H^+ from a base. One possibility is to assume the formation of a five-membered chelate involving N_7 and N_{10} in adenine; Hg^{II} might displace one H^+ from the amine and form a third Hg-Cl or Hg-OH coordination bond. But from the general tendency of Hg^{II} to form two collinear bonds, this structure seems less probable. If we assume that one Hg^{II} is bound to the adenine N_7, in the form of base-Hg-OH or base-Hg-Cl, then from the pH-stat titration result, we have to assume that the second Hg^{II} displaces concomitantly two H^+, and no plausible locus can be thought of.

Because the guanine-cytosine and adenine-thymine pairs involve different numbers of hydrogen bonds,[30] it seemed to be of interest to verify the effect of the base content on the binding of $HgCl_2$. The order of magnitude of the constant of dissociation, K, can be obtained from equation 2 by extrapolating to $(Cl^-) = 1$. As shown in Fig. 7, K increases in the order calf thymus (42% G-C), E. coli (50% G-C) and M. lysodeikticus (72% G-C), which indicates that the binding becomes

weaker as the guanine-cytosine content increases. This may be interpreted as being due to the fact that probably one Hg^{II} goes between guanine N_1 and cytosine N_1 and since guanine-cytosine pair involves three hydrogen bonds, this might hinder the binding of mercury. It is known that the T_m depends on the G-C content.

Since the "denaturation" consists mainly in the breakdown of hydrogen bonding between the base pairs of the two polynucleotide chains, this should enhance the affinity of DNA for Hg^{II}. This is in fact the case as can be seen in Fig. 7.

The foregoing discussions, rather speculative in nature, indicate the need for studies of Hg^{II} binding by nucleic acid subunits, such as the nucleosides, oligonucleotides and AT and GC polymers. Only with such information can an indication of the specific binding sites be derived.

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PROPOSITIONS

1. Preliminary experiments show a change in the ultraviolet absorption spectrum of $HgCl_2$ when acetate is added. No isosbestic point is observed which suggests the presence of the mixed complex Cl-Hg-Ac. Table given below indicates the potential one would obtain assuming various K's for the complex Cl-Hg-Ac, maintaining $(Cl^-)=1x10^{-4}F$ and considering the presence of $HgCl_2$, $HgAc_2$ and HgClAc complexes. The potential of the mercury electrode with respect to the reference electrode (S.C.E.) is given by $E = 593 + 29.7 \log (Hg^{++})$; K_A = the constant of formation of $HgCl_2 = 1.66x10^{13}$ (1); K_B = the constant of formation of $HgAc_2 = 1.66x10^9$ (assumed).

(Ac^-)	$(K_AK_B)^{1/3}$	$(K_AK_B)^{1/2}$	$2(K_AK_B)^{1/2}$	$5(K_AK_B)^{1/2}$
0 F	319 mv	319 mv	319 mv	319 mv
10^{-3}	318	317	317	314
10^{-2}	310	305	302	293
10^{-1}	263	259	257	254
1	201	201	200	199

Due to the small potential difference obtained among several K's assumed, the potentiometric analysis would not give a reliable value of the constant of formation of the mixed complex. The Spectrophotometry might be the method indicated in this case.

2. Although the existence of iron (III) phosphate com-

plex has been recognized for more than a century, the exact nature of the complex is still not definitely known, and in almost all of the work reported in literature, the formula of the complex has been assumed. (2). The only quantitative study of the Fe^{III} phosphate made is due to Lanford and Kiehl (3). They indicated the presence of $FeHPO_4^+$.

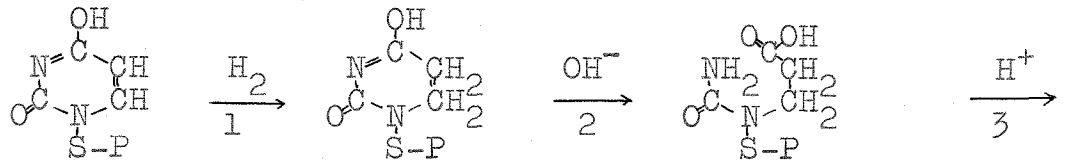
It was observed by spectrophotometric and potentiometric methods that the main complexes are $FeHPO_4^+$ and $Fe(HPO_4)(H_2PO_4)$ in the pH range 1-1.8, $(\Sigma Fe^{III}) \simeq 1x10^{-4}$-$1x10^{-3}$F, ($\Sigma$Phosphate) $\simeq 5x10^{-4}$- $1x10^{-2}$F(4). This work should be extended over a wider range of pH and phosphate concentration. Under these conditions, we should be able to find complexes involving triorthophosphate, OH^- and PO_4^{-3}. At high iron (III) concentration, phosphate complexes of polynuclear iron (III), -Fe-O-Fe-, are also expected to be formed.

All the complexes suggested in the past might exist under specific conditions, but a more quantitative study of the iron phosphate complexes is needed in order to obtain trustworthy values of the formation constants.

3. A method of preparing DNA and RNA deprived of pyrimidine bases (apyrimidinic acid) is proposed.

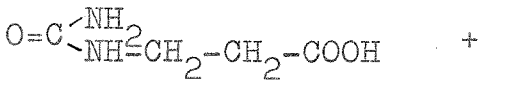
The stability of the N-glycosidic linkage of pyrimidine nucleotides to acid hydrolysis is due to the ethylenic unsaturation between the adjacent carbon atoms in the ring; reduction

of the 4,5-double bond destroys the resonating structure and renders the N-glycosidic linkage susceptible to acid hydrolysis. Cohn and Doherty (5) reported on the complete catalytic hydrogenation of the 4,5-double bond of pyrimidine nucleotides under mild conditions (1.0-1.5 atm of hydrogen with a 5% Rh on alumina catalyst; pH 2.0-5.0). The product is cleaved by dilute alkali at room temperature to give, e.g., in the case of uridylic acid, the N-ribose-phosphate of β-ureido propionic acid. Dilute acid hydrolyzes this substance to ribose phosphate and β-ureido propionic acid at room temperature.



uridylic acid

β-ureidopropionyl N-ribose-3'-phosphate



β-ureidopropionic acid

It should be possible to apply this method to both RNA and DNA in order to obtain nucleic acids deprived of pyrimidine bases. It is known from Chargaff's work (6) that nucleic acid (2% solution) at pH 1.6 and at 37°C, undergoes hydrolysis of the N-glycosidic linkage of the purine nucleotides. Therefore, apyrimidinic acid under slightly more drastic condition than that of step 3 should loose purine bases and lead to poly-

ribose-phosphate and polydeoxyribose-phosphate polymers. Zamenhof et. al. (7) reported the presence of the polyribose-3'-phosphate in the Hemophilus influenza, type b, but whether polydeoxyribose-phosphate exists in nature is not known.

4. It was suggested that the sequence p-Pu-p-Py is preferentially hydrolyzed by the pancreatic deoxyribonuclease (DNase I)(8) and that the minimal length of 3'-phosphoryl-terminated fragments susceptible to attack by DNase I is three nucleotide units (9).

$$\text{A-p-A-p-T-p} \longrightarrow \text{A-p-A} + \text{p-T-p}$$
$$\text{A-p-C-p} \not\longrightarrow$$

However, Sinsheimer (8) observed that the single exception is the case of cytidylic and guanylic acids. The occurence of dinucleotides in the digest of DNA with DNase I showed that the sequence guanylic-cytidylic is over five times as abundant as its isomer, indicating that in this case the sequence p-Py-p-Pu is preferentially hydrolyzed by the enzyme. de Garille et al. (10) confirmed Sinsheimer's observation but due to the small % of p-Pu-p-Py obtained in comparison to p-Pu-p-Pu, p-Py-p-Py and p-Py-p-Pu, they considered that 5'-terminated fragments may be fragments derived from the original termination of the chain. But this discrepancy may well be due to the presence of a different DNase not hitherto separated.

The cellulose ion-exchange chromatography has emerged

recently as a powerful tool for separation of proteins in single and complex biological mixtures. Mitz et al. (11) succeeded in isolating enzymes from solution and observed that enzymes could be kept in the exchanger without much loss in activity.

Since DNase is known to be unstable in aqueous solution, the use of cellulose exchanger might be a way of purifying it and also of keeping it in an active form. It is conceivable that DNase I, considered so far as a homogeneous enzyme, consists of two components and might be separated by cellulose ion-exchanger. If separated into two enzymes, each one should be tested for its specifity on oligonucleotides in order to clarify the discrepancy observed by Sinsheimer.

5. RNA is considered to have a secondary structure consisting of small helical regions that involve approximately half of its nucleotides (12). In aqueous solution it is in a rather contracted state similar to globular proteins (12).

It is proposed that when RNA is denatured, by heating or by adding denaturing substances, one should observe an increase in the viscosity due to the transition: contracted state $\longrightarrow$ random coil.

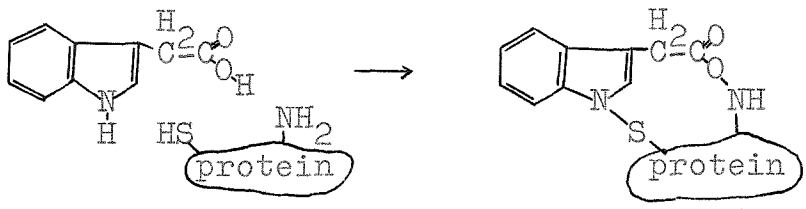
By a similar method used by Linderstrøm-Lang (13), one may be able to estimate the % of hydrogens involved in hydrogen-bonding. The existence of regions with helical configuration in insulin was shown by Linderstrøm-Lang by the following

method: (a) insulin is allowed to exchange all its hydrogens with deuterium by equilibrating with deuterium of D_2O; (b) insulin is then carefully dried; (c) resolution in ordinary water and the rate of appearance of deuterium, as measured by density change in the solvent, permits the division of total exchangeable atoms into those are instantaneously and slowly exchangeable. The slowly exchangeable hydrogens are involved in hydrogen-bonding and from the total number of hydrogens exchanged one can estimate the % involved in the helical configuration.

A direct application of the Linderstrøm-Lang method to RNA raises the problem of the solubility of RNA (step c). This difficulty can be overcome by using a modified method as follows: (a) RNA is initially dissolved in a small quantity of H_2O; (b) RNA solution is then transferred to D_2O; (c) samples are taken at every fixed interval of time and quickly lyophilized and carefully dried; (d) resolution in ordinary water and the amount of deuterium can be measured by density change in the solvent. In this way the number of slowly and instantaneously exchangeable hydrogens in RNA can be determined.

6. According to the current concept, auxin constitutes the prosthetic group of an enzyme; it must first combine with a specific protein to form an enzyme which then catalyzes a reaction essential to the growth process of plants. In sup-

port of this concept, it has been shown that free auxin is released from plant tissues and plant proteins by proteolytic agents (14), and that the kinetics of auxin action in Avena coleoptiles are best understood in terms of a two-point attachment of auxin to some acceptor, presumably protein (15).



An unambiguous confirmation of the existence of auxin-proteins in plant cells was reported by Galston (16). Indoleacetic acid fed to excised pea root tips is quickly and firmly bound to protein, approximately 0.1-1.0 mole of IAA being bound per mole of protein of assumed molecular weight of 10,000. Auxin is not removed from the protein by dilute acid or alkali, acetone or boiling.

Whether or not the auxin-protein described by Galston is the catalytic entity involved in the growth reaction is not known.

It is proposed that labeled synthetic auxins be used and be separated from plant tissue in the form of auxin-protein and reinjected into an another plant sample tissue in order to verify if this auxin-protein has catalytic activity or not. It is known that auxins can promote the differentiation of buds, the production of flowers, etc., and it may well be that each

activity is related to a particular kind of protein bound to auxin. This whole experiment is based on the premise that plant tissues absorb proteins. It would also be interesting to investigate the effect of the pyrimidine 5-fluorouracil on the production of the proteins involved in the auxin activities.

7. It has recently been reported (17) that Ribonuclease T_1 splits the internucleotide bonds between the 3'-guanylic acid groups and the 5'-hydroxyl groups of the adjacent nucleotides; Ribonuclease T_2 splits bonds between the 3'-adenylic acid groups and the 5'-hydroxyl groups of the adjacent nucleotides.

The specificities of T_1 and T_2 are rather interesting and it is proposed that

(a) RNases T_1 and T_2 should not require the presence of the pyrimidine bases for their enzymatic activities which solely depend on the purine bases. Therefore, RNases T_1 and T_2 should act on RNA deprived of the pyrimidine bases;

(b) Adenine has a primary amino group at C_6 and guanine has a primary amino group at C_2 and a hydroxo group at C_6. Therefore, the specifity of T_1 and T_2 depends on these two groups or two loci, and it would be of interest to verify their enzymatic activities on RNA treated with formaldehyde (this substance is known to react with p-amino groups) and also on RNA treated with HNO_2.

8. It is known that if two hydroxyl groups on adjacent carbon atoms in a ring compound have the cis configuration, boric acid forms a complex as shown below:

The end group of RNA satisfies the above requirement and therefore one may be able to obtain RNA dimer interconnected by borate, RNA-borate-RNA. Viscosity is expected to increase due to dimerization.

This complex might be useful in characterizing the terminal nucleotide in RNA. If it is a 3'-phosphoryl-terminated end group, dephosphorylation of the terminal nucleotide can be carried out with phosphomonoesterase. The formation of RNA dimer can be avoided by adding glucose with borate. 1 N NaOH at 20°C for 18 hours breaks phosphodiester internucleotide links of RNA at C-5' (18), leading to charged nucleotides and a neutral complex which can be separated and characterized.

9. The pH-stat titration carried out by adding Hg^{II} to calf thymus DNA solution (19) should be extended to DNA's of different guanine-cytosine contents and also to guanine-cytosine and adenine-thymine polymers. This information would greatly help to elucidate the binding site of Hg^{II} to DNA.

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