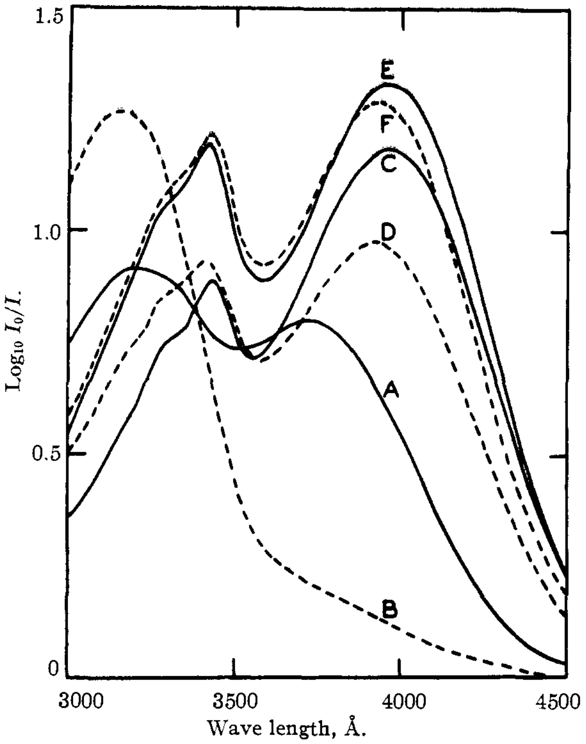

Fig. 2.—Absorption spectra of absolute ethanol solutions of typical chelates: A, $Sc(C_9H_6NO)_3 \cdot C_9H_6NOH$, 1.2 mg. Sc/l.; B, same as A with 10% water by volume; C, $Sc(C_9H_4Cl_2NO)_3$, 1.2 mg. Sc/l.; D, same as C with 20% water by volume; E, $Sc(C_9H_4Cl_2NO)_3 \cdot C_9H_4Cl_2NOH$, 1.2 mg. Sc/l.; F, same as E with 20% water by volume.

$Cl_2NO)_3$: 0.60–1.20 mg. Sc/l., $k_{\text{av.}}$ at 3430 Å. = 148.9, $k_{\text{av.}}$ at 3970 Å. = 191.3; $Sc(C_9H_4Cl_2NO)_3 \cdot C_9H_4Cl_2NOH$: 0.60–1.20 mg. Sc/l., $k_{\text{av.}}$ at 3430 Å. = 201.1, $k_{\text{av.}}$ at 3970 Å. = 220.6; $Sc(C_9H_4Br_2)_3 \cdot C_9H_4Br_2NOH$[13]: 0.60–1.20 mg. Sc/l., $k_{\text{av.}}$ at 3430 Å. = 217.2, $k_{\text{av.}}$ at 3990 Å. = 220.2; $Sc(C_9H_4ClINO)_3$, 0.60–1.20 mg. Sc/l., $k_{\text{av.}}$ at 3470 Å. = 160.3, $k_{\text{av.}}$ at 4020 Å. = 184.4; $Sc(C_9H_4ClINO)_3 \cdot C_9H_4ClINOH$: 0.50–1.00 mg. Sc./l., $k_{\text{av.}}$ at 3470 Å. = 215.3, $k_{\text{av.}}$ at 4020 Å. = 210.2.

Acknowledgment.—Funds received from an E. I. du Pont de Nemours and Company Grant-in-aid for partial support of this investigation are gratefully acknowledged.

[13] $Sc(C_9H_4Br_2NO)_3$ was too difficultly soluble in ethanol to permit study.

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The Interaction of $HgCl_2$ with Sodium Thymonucleate

BY C. A. THOMAS*

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An interesting reaction of sodium thymonucleate (DNA) was investigated by Katz,[1] who found that $HgCl_2$ undergoes a reversible combination with DNA which results in a large increase in the molecular weight as determined by light scattering. Although the identity of the combining species has not been determined, we will tentatively assume for simplicity in this discussion that it is the $HgCl_2$ molecule. Katz further found that the addition of Cl^- or CN^- would completely reverse the complexation leaving free DNA. He interpreted the large increase in the molecular weight that was brought about by the addition of $HgCl_2$, as being the result of partial aggregation. However, he assumed that the $HgCl_2$–DNA complex had the same refractive index increment (dn/dc) as free DNA, while it is probable that the complex has a higher dn/dc value than the free DNA. This would cause his reported molecular weights of the complex to be somewhat too high. With this in mind, partial aggregation need not be postulated.

Further investigation of this reversible complexing reaction has revealed that the ultraviolet absorption spectrum of DNA is substantially altered by the addition of $HgCl_2$. In 0.40 *M* acetate buffer, *p*H 5–6, free DNA has an absorption maximum at 258 mμ. On the addition of $HgCl_2$ (dissolved in the same buffer) the absorption maximum shifts over to 275 mμ. The addition of NaCl to this solution will cause the absorption maximum to again shift back to 258 mμ. This, together with Katz' observations, is strong indication that the DNA–$HgCl_2$ complex is reversed by the addition of NaCl.

On examining the shift of the absorption spectrum more carefully, we find that all curves go through an isosbestic point located at 238.5 mμ (see Fig. 1). Defining r = total moles of added $HgCl_2$/moles P, we find that all curves for which r is less than 0.60 pass through isosbestic points at 238.5 and 262.5 mμ. On increasing the mercury concentration from r = 0.60 to 10, the absorption curves display a new isosbestic point at 274.5 mμ but still pass through the first one at 238.5 mμ.

This behavior suggests in this particular case that the reaction proceeds by at least two steps and that the first reaction is essentially complete before the second reaction begins. If this is the case, the curves in Fig. 1 which pass through the isosbestic points at 238.5 and 262.5 mμ can be considered to result from two components in equilibrium with each other and absorbing ultraviolet light independently.

Choosing two appropriate wave lengths, it is possible to write two simultaneous equations for the total optical density at these wave lengths in terms of the concentration of the free DNA and the concentration of the complex. In this case we have chosen wave lengths of 257.5 and 271.0 mμ because they are near maxima, and yet as far from isosbestic points as possible; the results do not depend on this choice.

$$D_{257.5} = E^{\text{DNA}}_{257.5}\, C^{\text{DNA}} + E^{\text{complex}}_{257.5}\, C^{\text{complex}}$$

$$D_{271} = E^{\text{DNA}}_{271}\, C^{\text{DNA}} + E^{\text{complex}}_{271}\, C^{\text{complex}}$$

Employing Chargaff's value of 6650 ± 50 (at 259 mμ in the presence of salt) for the extinction coefficient with respect to phosphorus (E(P)),[2] it is

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[1] S. Katz, THIS JOURNAL, **74**, 2238 (1952).

[2] E. Chargaff and R. Lipshitz, *ibid.*, **75**, 3658 (1953).

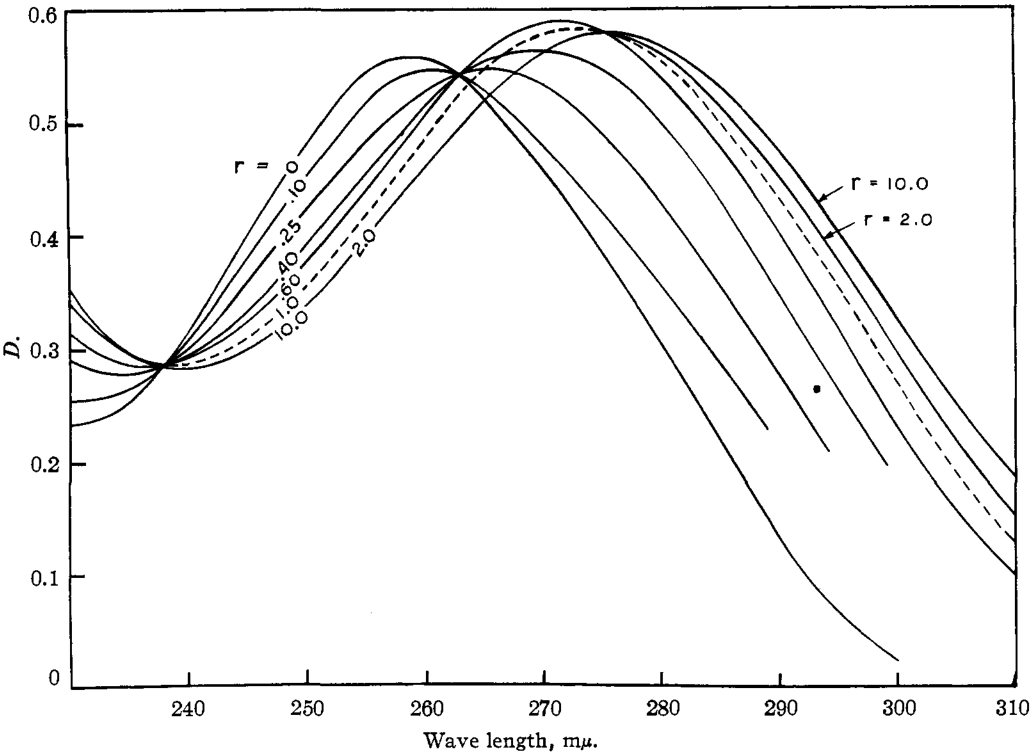

Fig. 1.—The influence of $HgCl_2$ on the ultraviolet absorption spectra of DNA. *D* is defined as the "optical density" or $\log_{10} I_0/I$. Spectra of DNA in 0.40 *M* acetate buffer, *p*H 5-6 with increasing *r*, *r* = moles $HgCl_2$/grundmoles DNA.

possible to calculate all of the other extinction coefficients in the above equations. Solving for the concentration of the DNA-$HgCl_2$ complex, we find that 0.56 ± 10% moles of $HgCl_2$ complex per mole of DNA phosphorus. Furthermore, the equilibrium is well in favor of the complex form. The stoichiometry of the second reaction cannot be measured in this manner because the shift is too small. But it is clear that the equilibrium constant is not as large as in the first reaction.

The character of the DNA precipitate which results from the addition of ethanol to DNA solutions in 0.40 *M* acetate buffer, depends very markedly on the amount of $HgCl_2$ present. In the absence of $HgCl_2$, the ethanolic precipitate is fibrous and can be spooled up about a stirring rod. When 0.5 mole of $HgCl_2$ have been added per each mole of phosphorus, the character of the precipitate is a fine powder which settles slowly. X-Ray diffraction patterns of these two types of precipitates reveal that the $HgCl_2$–DNA precipitate is very amorphous while the ethanolic precipitate of the original DNA is highly crystalline, displaying 6 well-defined diffraction rings.

Preliminary experiments show that the sedimentation constant (measured with about 0.2% DNA in 0.40 *M* acetate buffer *p*H 5–6) increases in a linear fashion with increasing amounts of $HgCl_2$. For instance, when $r = 0$, $s_{20} = 13$. On increasing r to $r = 3$, s_{20} becomes equal to 50. The intrinsic viscosity of the complex at an average gradient of 1000 $sec.^{-1}$ is about 9 compared to a value of 20 for the original DNA under the same conditions. The apparent gradient dependence of viscosity of the original DNA is decreased by a factor of 5. All of these observations are in accord with the decrease in size of the DNA molecule on the addition of $HgCl_2$ observed by Katz.

Discussion.—Since the purine and pyrimidine residues of DNA are primarily responsible for the absorption maxima at 260 mμ, the shift in the absorption spectra brought about by the addition of $HgCl_2$ strongly suggests that the binding occurs at these bases. Although the nature of this combination is unknown, it is possible that the $HgCl_2$ is coördinating with the conjugated double bond systems in guanine, cytosine and thymine much in the same manner that $HgCl_2$ can combine with mesityl oxide.[3] Another possibility is that the $HgCl_2$ combines with NH_2 groups in adenine, guanine or cytosine. In the Crick–Watson[4] model for DNA, these bases are involved in hydrogen bonds which hold the two polynucleotide strands together. It is likely that when these bases combine with $HgCl_2$ certain hydrogen bonds between the polynucleotide chains will be destroyed. This destruction would result in a more flexible molecule which would then spontaneously assume a more compact configuration. This is in agreement with the decrease in size observed by light-scattering[1] and viscosity measurements. Since the original size and shape of the DNA molecule is restored after the $HgCl_2$ is removed, a sufficient number of hydrogen bonds must remain intact in the presence of $HgCl_2$ to provide a "skeleton" to guide the reformation of the molecule when the reaction is reversed.

It is highly probable that there is some inter-

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[3] J. Chatt, *Chem. Revs.*, **48**, 7 (1951); see pp. 11.

[4] J. D. Watson and F. H. C. Crick, *Nature*, **171**, 747 (1953).

action between the phosphate groups and the mercuric ions Hg^{++} and $HgCl^{+}$ about which we have no information.

Acknowledgment.—The author is grateful for the help and advice of Dr. Sidney Katz.

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The Photochemical Formation of Ozone. Foreign Gas Effects on the Mercury Sensitized Reaction at 2537 Å. and the Unsensitized Reaction at 1849 Å.

BY DAVID H. VOLMAN

RECEIVED AUGUST 16, 1954

Following the formation of $Hg(^3P_1)$ atoms by adsorption of mercury resonance radiation, 2537 Å., three processes ultimately leading to ozone formation may be considered[1,2]

$$Hg^* + O_2 \longrightarrow Hg + 2O,\ \Delta H = 5.5 \text{ kcal.} \quad (1)$$

$$Hg^* + O_2 \longrightarrow HgO(g) + O,\ \Delta H = -87 + \lambda \quad (2)$$

$$Hg^* + O_2 \longrightarrow Hg + O_2^* \quad (3)$$

where λ is the heat of sublimation of HgO(s). The calculations are based on $Hg(^3P_1)$ atom but the essential features would not be changed by considering instead $Hg(^3P_0)$. The activated oxygen species may be either normal oxygen molecules in high vibrational states or electronically excited oxygen.[3]

Calculations from collision theory based on the endothermicity of eq. 1 indicate that only one collision in about 10^4 would be effective. Since the quantum yield for the reaction is of the order of 0.03,[4] and since long chains are not possible, it appears unlikely that this reaction is an important one.

The value of λ for eq. 2 is not known but Noyes[5] estimates that it is not over 23 kcal. Thus (2) is exothermic, and therefore (2) and (3) are both possible paths. Dickinson and Sherrill[6] have shown that at least 7 molecules of ozone are formed for each mercury atom passing through the reaction zone. Volman[4] has evidence that this value may actually be considerably higher. Since the formation of ozone from oxygen is 34 kcal. endothermic, a single activated mercury molecule can at best account for 3 ozone molecules. This could possibly be as high as 4 if the heat evolved in the formation of HgO were available. Even these yields are unlikely since mechanisms giving these values do not appear probable. A postulated solution to this dilemma is the reaction[3]

$$HgO(g) + O_2 \longrightarrow Hg + O_3$$

However this reaction is endothermic to the extent of 68-λ kcal.

The troublesome features of (1) and (2) are largely obviated in (3). Activated oxygen produced may react by

$$O_2^* + O_2 \longrightarrow O_3 + O \quad (4)$$

followed by

$$O + O_2 + M \longrightarrow O_3 + M \quad (5)$$

Equation 5 is common to mechanisms involved in (1), (2) and (3) since O atoms are postulated for all three.

It was felt that a study of the effects of foreign gases in the reaction system would give information relative to the mechanism of ozone production since foreign gases would play a role in the following processes

$$Hg^* + M \longrightarrow Hg + M^* \quad (6)$$

$$O_2^* + M \longrightarrow O_2 + M^* \quad (7)$$

as well as in eq. 5 above. In the course of the experiments, it became evident that information on the effects of foreign gases on the unsensitized reaction would also be desirable.

Experimental

The experimental flow method used was similar to that described earlier.[7] High purity commercial gases from cylinders were measured by flow meters and dried over magnesium perchlorate. The light source was a mercury–rare gas low pressure discharge tube in the shape of a helix, 70 mm. across and 250 mm. long. The light emitted from such a lamp is primarily a mixture of the 1849 and 2537 Å. mercury resonance lines. Since the 1849 Å. line will not be transmitted through a few mm. of liquid water,[8] it is possible to prevent the short wave length radiation from reaching the reaction zone. For the unsensitized reaction, the lamp and reaction tube, 30 mm. diam., were both surrounded by air. Under these conditions ozone was formed in the absence of mercury vapor. For the sensitized reaction, the reaction vessel, 20 mm. diam., and surrounding lamp were contained in a water-bath thermostated at 45°, the temperature for optimum light intensity.[8] Under these conditions ozone was not formed in the absence of mercury vapor but was formed in the presence of mercury vapor. The pressure of mercury vapor entering the reaction zone was adjusted to a value corresponding to saturation at 20° in the manner described previously.[7] Since oxygen gas at atmospheric pressure can absorb only negligible amounts of light of wave length 2537 Å., the formation of ozone in the unsensitized reaction may be attributed primarily to the absorption of the 1849 Å. resonance line by oxygen molecule. Ozone in the effluent gases was determined iodimetrically after absorbing in a neutral potassium iodide solution.

Results and Discussion

Number of Cycles Involving Mercury Vapor.—In the sensitized reaction a red-orange deposit of mercuric oxide was observed in agreement with the results of Dickinson and Sherrill.[6] This deposit was heaviest at the entrance to the reaction zone and tapered off so that no deposit was observable after about 8 cm. The effluent gas was found to be free of mercury vapor. From the yield of ozone and the vapor pressure of mercury entering the reaction zone, a value for the number of ozone molecules formed for each mercury atom entering the reaction zone may be determined. For example, at a flow rate of 1.0 liters oxygen per minute, the yield of ozone was 4.2×10^{-6} mole per minute. The gas stream contained mercury equivalent to saturation vapor pressure at 20.0°. Thus for each mercury atom about 60 molecules of ozone were

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[1] Other processes involving postulated but not established molecular species have sometimes been considered, *i.e.*, HgO_2. *Cf.* W. A. Noyes, Jr., THIS JOURNAL, **49**, 3100 (1927).

[2] Thermochemical values from F. R. Bichowsky and F. D. Rossini, "The Thermochemistry of Chemical Substances," Reinhold Publ. Corp., New York, N. Y., 1936.

[3] W. A. Noyes, Jr., and P. A. Leighton, "The Photochemistry of Gases," Reinhold Publ. Corp., New York, N. Y., 1941, p. 225.

[4] D. H. Volman, *J. Chem. Phys.*, **21**, 2086 (1953).

[5] W. A. Noyes, Jr., THIS JOURNAL, **53**, 514 (1931).

[6] R. G. Dickinson and M. S. Sherrill, *Proc. Natl. Acad. Sci.*, **12**, 175 (1926).

[7] D. H. Volman, *J. Chem. Phys.*, **14**, 707 (1946).

[8] L. J. Heidt and H. B. Bayles, THIS JOURNAL, **73**, 5728 (1951).