[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

# The Reversible Reaction of Sodium Thymonucleate and Mercuric Chloride

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The sodium salt of calf thymus nucleic acid is found to react with mercuric chloride to produce what is, essentially, an incomplete mercuric salt of nucleic acid. Two of the physical manifestations of the reaction are a decrease in viscosity and an increase in turbidity. These have been studied by appropriate methods. The reaction is accompanied by aggregation (but not precipitation) and can be completely reversed in solution as demonstrated for the first time by light scattering techniques. Data from binding experiments have been combined with those from light scattering measurements to give a consistent picture of the mechanisms involved.

## Introduction

The investigations of Hammarsten[2] in 1924 first demonstrated that various neutral inorganic salts decrease the viscosity of solutions of the sodium salt of desoxyribonucleic acid (hereafter SDNA). Hammarsten found that the effects were due chiefly to the cations and that the salts were most effective in the concentration range 0 to $1 \times 10^{-2}$ $N$, the viscosity decreasing relatively little with higher salt concentration. Similar results have been obtained in more recent investigations.[3-7]

The present study arose from the observation that as little as $1 \times 10^{-3}$ $M$ mercuric chloride produced an easily measurable viscosity decrease in buffered SDNA solutions already 0.3 $N$ in sodium ion. The techniques of viscosity, light scattering, equilibrium dialysis and electrophoresis have been applied to the reaction to examine its extent, reversiblity and mechanism.

## Preparation and Treatment of SDNA

The SDNA was prepared by the improved method used by Signer and Schwander[8] for their preparation VIII with only minor changes from their procedure. The product was redissolved in water and reprecipitated with ethanol in an effort to remove excess sodium chloride. Following Signer, the Weber modification of the Sakaguchi reaction was employed as a test for arginine-containing protein. The test gave a light yellowish coloration as described by Signer for his preparation VIII, instead of the reddish coloration obtained from proteins containing arginine. According to Signer,[8] this indicates a basic protein content of 0.2% or less. The SDNA was stored over saturated sodium chloride solution[5] at 2°; after two months equilibration it was found to have a water content of 27.1% determined by drying samples of about 0.1 g. to constant weight in a vacuum oven at 75°. For comparison with the preparations of Signer and his co-workers, the SDNA was characterized by measurement of its relative viscosity in an Ostwald-Cannon viscometer with an average velocity gradient of 910 $sec.^{-1}$ calculated by the formula of Kroepelin.[9] At a concentration of 0.0149% SDNA in 10% sodium chloride at $p$H 6.0$_0$, the relative viscosity at 20° was $1.25_4$; this may be compared with $1.25_7$ for preparation VIII and 1.27 for preparation VII of Signer and co-workers under the same conditions, obtained by graphical interpolation of their data.[8]

Solutions of SDNA were usually made by adding weighed amounts to a known volume of water or buffer, taking the water content of the SDNA into account. The additional volume contributed to the solution by the SDNA was negligible in all cases and no corrections were made for it. In some cases, solutions were made by weighing out appropriate amounts of a stock solution (about 1%) whose concentration was determined by the dry weight of a weighed aliquot. Weighing was employed rather than volumetric delivery because of the very high viscosity and jelly-like character of this solution.

The buffer employed throughout was composed of 0.10 $M$ sodium citrate and 0.001 $M$ citric acid giving a $p$H of 6.9$_0$ and an ionic strength of 0.60 mole per liter. The salt–acid ratio of the buffer is such as to make its buffering action small, but the citrate system at this $p$H was nevertheless used for the following reasons: (1) it was considered desirable to maintain a $p$H near neutrality to avoid possible acidic or basic degradation[10-12] of the SDNA; (2) citrate, even in 0.01 $M$ solution, inhibits the desoxyribonuclease system[13]; (3) unlike other buffer systems (*e.g.*, phosphate), it did not immediately precipitate insoluble mercury salts. After two or three weeks at room temperature, however, the citrate buffer will, if mercuric chloride has been added, show a scanty white precipitate presumably of mercurous chloride.[14] For this reason mercuric chloride was added to the buffer solution just before use. It was observed that buffer–mercuric chloride solutions containing SDNA in addition showed no precipitate even after two or three months at room temperature and that buffer–mercuric chloride solutions used in light scattering did not disclose any unusually high turbidities after centrifugation.

**Concentration Units.**—For our purposes it is most convenient to give the concentration of mercuric chloride in an SDNA solution as the number of moles of mercuric chloride per mole of nucleotide. This quantity, which will be called $r$, is based on the assumption of a polynucleotide formula of $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$. The formula assumes, on the average, equimolar quantities of adenine, cytosine, guanine and thymine and gives an average nucleotide weight of 326.

## Qualitative Observations

The preparation of SDNA used in these experiments has the characteristics of high molecular weight preparations. A very fibrous precipitate is obtained by alcohol precipitation and the water solutions show visible rigidity and gel-like behavior even at a concentration of 0.3%. When mercuric chloride is added in sufficient amounts, the rigidity of the solution vanishes. As judged visually, the rigidity as well as the viscosity can be restored by addition of sodium chloride, bromide, iodide and cyanide. If an attempt is made to precipitate the mercuric chloride treated SDNA from solution, it is found that a higher alcohol concentration is necessary and that the precipitate itself has lost most of its fibrous character, appearing more

(1) du Pont Postdoctoral Fellow in Chemistry, University of Wisconsin, Madison, Wisconsin, 1950–1951.

(2) E. Hammarsten, *Biochem. Z.*, **144**, 383 (1924).

(3) J. P. Greenstein and W. V. Jenrette, *Cold Spring Harbor Symposia Quant. Biol.*, **6**, 109 (1938).

(4) A. Wissler, Dissertation, Univ. Bern, 1941.

(5) A. Knapp, Dissertation, Univ. Bern, 1946.

(6) R. M. Creeth, J. M. Gulland and D. O. Jordan, *J. Chem. Soc.*, 1141 (1947).

(7) R. Signer and H. Schwander, *Helv. Chim. Acta*, **32**, 854 (1949).

(8) R. Signer and H. Schwander, *ibid.*, **33**, 1522 (1950).

(9) H. Kroepelin, *Kolloid Z.*, **47**, 294 (1929).

(10) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 1806 (1943).

(11) J. M. Gulland, D. O. Jordan and H. F. W. Taylor, *J. Chem. Soc.*, 1131 (1947).

(12) L. E. Krejci, L. Sweeney and J. Hambelton, *J. Franklin Inst.*, **248**, 177 (1949).

(13) M. McCarty, *J. Gen. Physiol.*, **29**, 123 (1946).

(14) J. W. Mellor, "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. IV, Longmans, Green and Co., London, 1923, p. 834.

flocculent. Solutions in which the viscosity has been restored by the reagents mentioned, however, again yield a fibrous precipitate on the addition of alcohol.

A solution of SDNA may be saturated with mercuric chloride at room temperature (approximately 0.27 *M* at 25°) without precipitation but small amounts of mercuric nitrate and somewhat larger quantities of mercuric acetate will precipitate SDNA from solution.

## Viscosity Measurements

A simple Ostwald viscometer of the Cannon–Fenske[15] modification was used for semi-quantitative observations of the effect of mercuric chloride on the viscosity of SDNA solutions. The interpretation of viscosity measurements of SDNA solutions is complicated by the fact that these solutions show marked non-Newtonian behavior.[3,6,7,16] The results were therefore used only as a guide to quantitative experiments. For this reason, no kinetic energy corrections were made for the viscometer even though its flow time for water was only 10.6 seconds and no attempt was made to interpret the results on a molecular basis.

A concentration range of $r = 0$ to $r = 4.9$ in mercuric chloride was covered in the experiment. The SDNA concentration was kept constant at 0.033%. The *p*H of the solutions varied from $6.8_8$, for the solution containing no mercuric chloride, to $6.7_0$ for the solution in which $r = 4.9$, owing to the slight hydrolysis of mercuric chloride. It is known,[6] however, that the viscosities of SDNA solutions are constant *p*H 5.8 and 11, so the viscosity decrease cannot be attributed to the small *p*H change with $r$.

In Fig. 1, which gives the results of these measurements, $\eta/\eta_0$ is the ratio of the outflow time of the SDNA, buffer, mercuric chloride solution to the outflow time of the buffer solution. A relatively large initial drop in viscosity with $r$ is observed which begins to level off at about $r = 1$. Although these changes are small compared to those observed when increasing amounts of simple neutral salts are added to initially salt free SDNA, they are significant because the mercuric chloride concentration is so low compared to that of the buffer.

## Light Scattering Measurements

**Apparatus and Procedure.**—The instrument used for these studies makes use of the opal diffusor working standard method devised by Brice, Halwer and Speiser.[17] The light source is an AH-3 mercury vapor lamp stabilized by an electronic voltage regulator. The detector is an RCA-1P21 tube powered by batteries. Its unamplified current is fed directly to a high sensitivity box galvanometer (Leeds-Northrup model 2430-d). The photomultiplier tube is mounted in a housing which contains the optical system suggested by Zimm[18] for maintaining an image of constant size on the photocathode. The housing is mounted on an arm which can be rotated from 0 to 135° about a platform and cell table on which cells of various designs can be placed. The cell used for these studies was hand blown and resembles a 50-ml. erlenmeyer flask with a neck of increased length and diameter so that dilutions can be made in the cell. The cell geometry was checked by measuring the radial distribution of intensities from a weak solution of fluorescein. When corrected for the water blank, the cell was found usable from 25 to 135° with corrections of 0 to 3%.

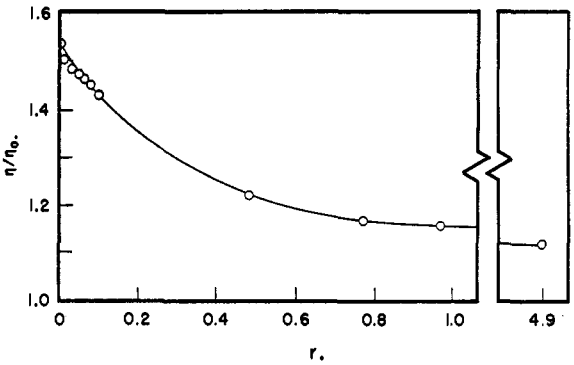


Fig. 1.—Relative viscosities of 0.0331% SDNA at various concentrations of mercuric chloride, *p*H 6.9 to 6.7.

The instrument was calibrated with a solution of polystyrene in butanone, the polystyrene being a standard sample kindly furnished by Professor P. Debye of Cornell University. The value of Carr and Zimm[19] for the reduced intensity at 90° and a wave length of 436 mμ was used in the calibration.

Solutions containing SDNA in buffer were centrifuged approximately 16 hours at 20,000 times gravity to remove particulate matter. For those solutions containing mercuric chloride in addition, centrifuging for approximately an hour at 20,000 times gravity was found adequate presumably because of the decreased viscosity of the solutions. The intensity of scattering from the solvent at angles from 25 to 135° was first measured and the solvent was then concentrated in SDNA by successive additions of the centrifuged SDNA in buffer. The lowest angle from which extrapolations of angular data were made was 25°, except in the case of Fig. 10, in which the lowest angle was 30°.

All glassware employed in the light scattering measurements was previously cleaned with dichromate–sulfuric acid cleaning solution and rinsed several times with water redistilled from an all-glass still. The cellulose nitrate centrifuge tubes were cleaned with a detergent and also rinsed with redistilled water before drying.

**Results in the Absence of Mercuric Chloride.**—A Zimm plot[18,20] of the data for SDNA in citrate buffer at *p*H $6.8_9$ (Run 1) is shown in Fig. 2. The concentration of the SDNA is $c$ grams per ml.; $K$ is equal to

$$\{2\pi^2 n^2 (dn/dc)^2\}/\lambda_0^4 N_0$$

where $n$ is the refractive index of the solution, $\lambda_0$ is the wave length of the incident light in vacuum, (436 mμ), and $N_0$ is the Avogadro number. The reduced intensity is given by

$$R_{\theta u} = (i_\theta r^2)/I_{ou}(1 + \cos^2\theta)$$

The symbol $R$ is used here in the sense of Edsall and Dandliker[21] to designate that both the horizontal and vertical components in the scattered light are measured, the subscript u indicating unpolarized incident light and $\theta$ the scattering angle. The other symbols are: $i_\theta$, the scattered intensity per unit volume of solution at angle $\theta$; $I_{ou}$, the intensity of the unpolarized incident beam and $r$, the distance from the scattering center to the radiation detector.

(15) M. R. Cannon and M. R. Fenske, *Ind. Eng. Chem., Anal. Ed.*, **10**, 297 (1938).

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(17) B. A. Brice, M. Halwer and R. Speiser, *J. Optical Soc. Am.*, **40**, 768 (1950).

(18) B. H. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

(19) C. I. Carr and B. H. Zimm, *ibid.*, **18**, 1616 (1950).

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(21) J. T. Edsall and W. B. Dandliker, *Fortschr. Chem. Forschung*, **2**, 1 (1951).

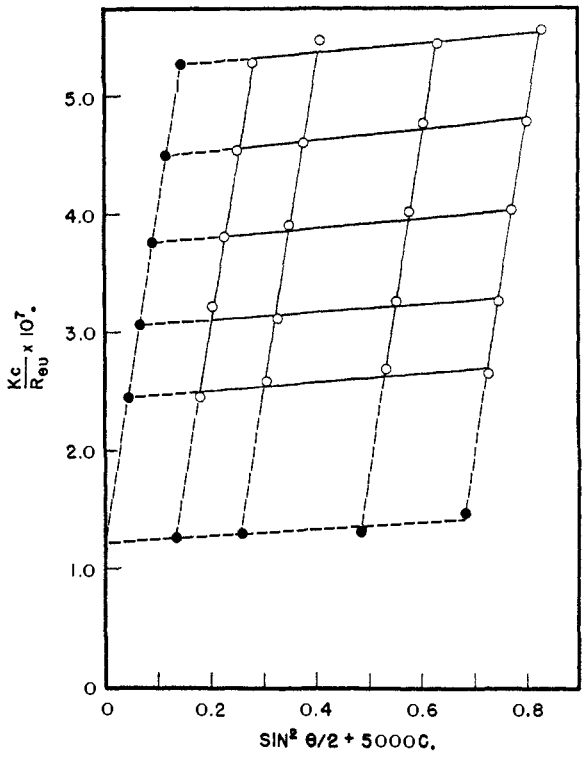


Fig. 2.—Zimm plot for SDNA in 0.101 $M$ citrate buffer, $p$H 6.9, in absence of mercuric chloride; run 1.

The intercept of the double extrapolation in Fig. 2 (to zero angle and zero concentration) is the reciprocal of the weight average molecular weight of the SDNA. The value obtained is $8.0_6 \times 10^6$ for this preparation, using a $dn/dc$ of 0.160 ml. per gram.[22] It must be emphasized that this result for the molecular weight may not be taken to characterize SDNA preparations generally as it is well known that the molecular weight of SDNA depends markedly on the method of preparation, $p$H and, possibly, the concentration of neutral salts.

Figure 3 is a plot of the reciprocal of the particle scattering factor,[23] $P(\theta)$, as a function of angle. This factor may be defined by the equation[18] $Kc/R_{\theta u} = 1/M_w P(\theta) + 2A_2c$. It is the scattering at angle $\theta$ relative to that at 0° and has been computed for several geometric models including spheres, rods and coils.[24,25] The other quantities are $M_w$, the weight average molecular weight of the solute and $A_2$, the second virial coefficient correcting for non-ideality.

The theoretical curves for rods and coils are compared with experimental results in Fig. 3, from which it is apparent that neither of the models fits the experimental data. The calculated curve for spheres has not been included because it lies well above that for coils. Qualitatively, these results are in excellent agreement with those previously obtained by Miss B. H. Bunce[26] in P. M. Doty's laboratory on an SDNA preparation of

(22) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).

(23) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **18**, 1211 (1950).

(24) B. Zimm, R. S. Stein and P. Doty, *Polymer Bull.*, **1**, 90 (1945).

(25) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).

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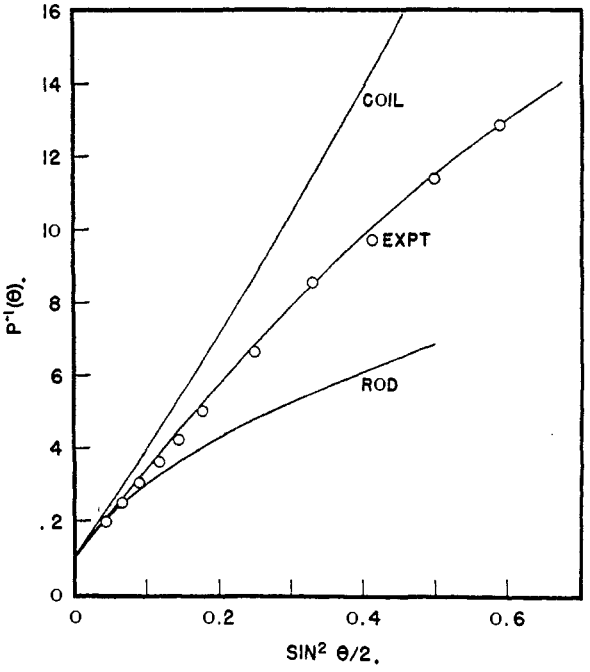


Fig. 3.—Reciprocal particle-scattering factors for SDNA in 0.101 $M$ citrate buffer, $p$H 6.9, in absence of mercuric chloride; run 1.

Gulland[27] and co-workers. Both experimental inverse particle scattering factors lie between the theoretical for rods and coils and both show a gentle downward slope at higher angles.

**Results in the Presence of Mercuric Chloride.**—Figures 4 and 5 show the results obtained by light scattering of SDNA in buffer with added mercuric chloride. In this experiment (Run 2) the ratio of mercuric chloride was kept constant during dilution at $r = 1.49$. It will be noted from Fig. 1 that this value of $r$ is in the region where further addition of mercuric chloride produces little additional decrease in viscosity. The molecular weight obtained in this case is $15.9 \times 10^6$ and, as before, the experimental particle scattering curve lies between the theoretical curves for rods and coils.

In Run 3, Figs. 6 and 7, the mercuric chloride concentration was kept constant at $3.07 \times 10^{-3}$ $M$ while the SDNA concentration was varied so that extrapolation to infinite dilution of SDNA represents an extrapolation in which $r$ becomes very large. It is evident from the figures that essentially the same results were obtained in Run 3 as in Run 4. What at first appears to be an increased slope, $A_2$, in Fig. 6 will shortly be seen to be an increasing molecular weight as $r$ increases.

Table I summarizes the data for Runs 1 to 3 and includes the values of $L$, the length of a rod,

TABLE I

MOLECULAR CONSTANTS DERIVED FROM LIGHT SCATTERING DATA

| Run | $r$ | $p$H | $M_w \times 10^{-6}$ | $L$, mμ | $R$, mμ |
|---|---|---|---|---|---|
| 1 | 0 | 6.89 | 8.06 | 735 | 520 |
| 2 | 1.49 | 6.66 | 15.9 | 644 | 456 |
| 3 | $\rightarrow\infty$ | 6.65 | 16.0 | 616 | 436 |

(27) J. M. Gulland, D. O. Jordan and C. J. Threlfall, *J. Chem. Soc.*, 1129 (1947).

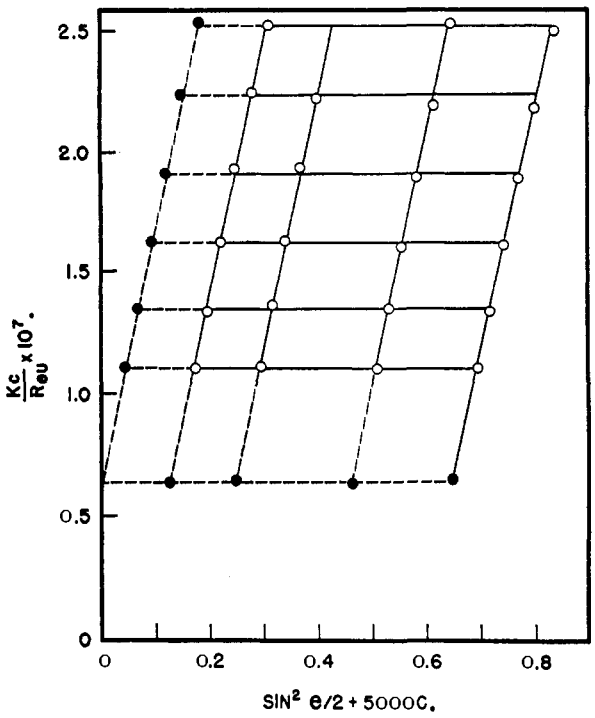


Fig. 4.—Zimm plot for SDNA in 0.101 *M* citrate buffer; *p*H 6.8, $r = 1.49$ throughout dilution; run 2.

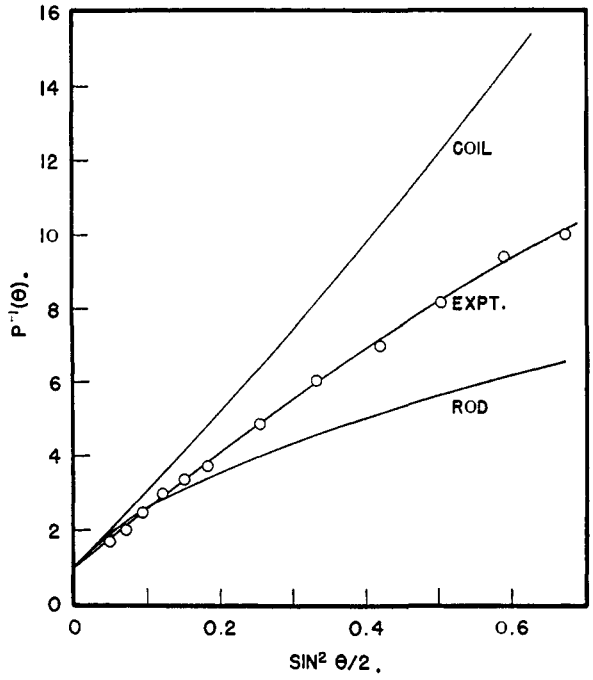


Fig. 5.—Reciprocal particle scattering factors for SDNA in 0.101 *M* citrate buffer, *p*H 6.8, $r = 1.49$ throughout dilution; run 2.

and $R$, the root mean square distance between the ends of a random coil, obtained by assuming these models for purposes of estimation. The values were calculated from the slopes and intercepts of the Zimm plots as described by Zimm.[18]

It is evident that the over-all molecular size of SDNA decreases in mercuric chloride solutions of these values of $r$, although qualitative differences

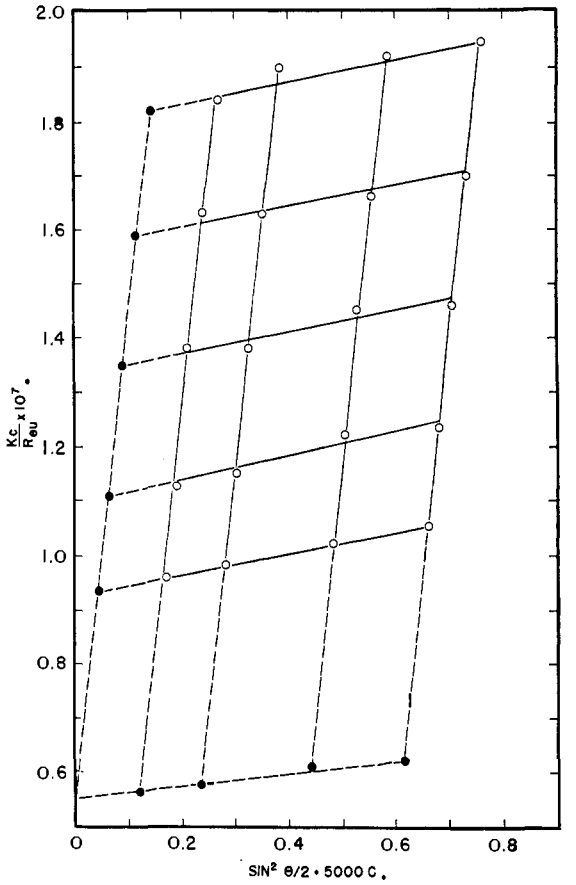


Fig. 6.—Zimm plot for SDNA in 0.101 *M* citrate buffer at constant mercuric chloride concentration of $3.07 \times 10^{-3}$ *M*; run 3.

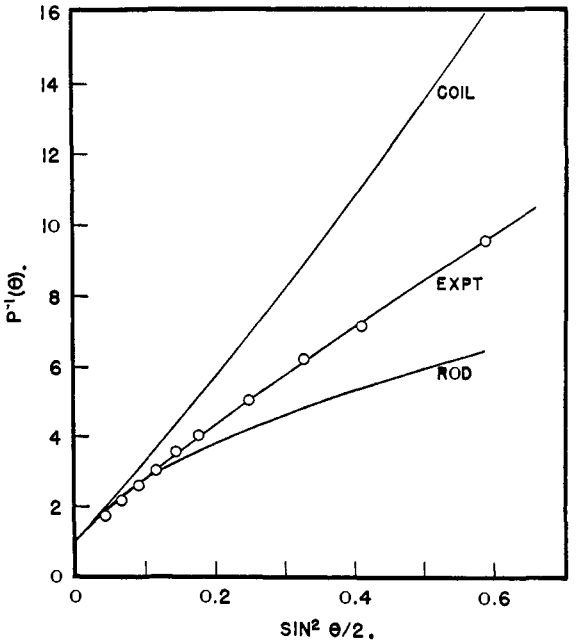


Fig. 7.—Reciprocal particle scattering factors for SDNA in 0.101 *M* citrate buffer at constant mercuric chloride concentration of $3.07 \times 10^{-3}$ *M*; run 3.

between the particle scattering factor curves for treated and untreated SDNA are not marked.

The effect of a wide range of $r$ on the molecular

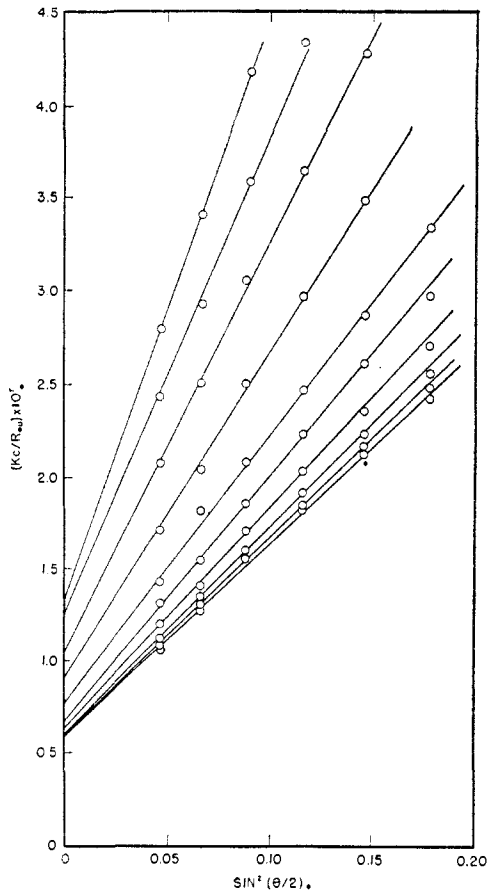


Fig. 8.—Angular scattering curves for SDNA with increasing $r$. The values of $r$, starting from the topmost curves, are: 0.00, 0.096, 0.193, 0.326, 0.520, 0.904, 1.29, 2.26, 3.21, 4.16; run 4.

weight of SDNA was obtained in an experiment (Run 4) in which increasing amounts of mercuric chloride in buffer were added to a fixed amount of buffered SDNA. The SDNA concentration before the addition of mercuric chloride was 9.36 $\times$ $10^{-5}$ g./ml. and the range $r$ = 0 to $r$ = 4.2 was covered in this experiment. It may be seen from Figs. 2 and 4 that the concentration dependence of $Kc/R_{\theta u}$ is slight in the first case and approximately zero in the second. Molecular weights may, therefore, be obtained from the reduced intensities at finite concentration provided a suitable correction is made for the concentration dependence. In Run 4, Fig. 8, the values of $Kc/R_{\theta u}$ obtained after each addition of mercuric chloride were plotted *vs.* $\sin^2(\theta/2)$ yielding good straight lines from 25° to 50°. The values extrapolated to $\theta$ = 0° represented $(1/M_w) + 2A_2c$. For simplicity's sake, values of $A_2$ were determined by assuming that $A_2$ decreased linearly with $r$ from $r$ = 0 to 1.49 and was equal to zero for $r \geq 1.49$, making use of the slopes obtained in runs 1 and 2. If the charge on the molecule were known, it would be possible to estimate the change in $A_2$ more accurately.[28] This is an unnecessary complication, however, in view of the smallness of the correction involved, the maximum correction being $-8\%$ in $Kc/R_{\theta u}$ for the solution containing no mercuric chloride. The Zimm plots also show that the lines of angular dependence of $Kc/R_{\theta u}$ are parallel to each other, indicating that $P(\theta)$ is independent of concentration in the range studied here. This permits calculation of lengths (based on the rod and coil models) at finite concentration. Figure 9 shows the results obtained in this experiment with respect to molecular weights and lengths calculated for both the rod and coil models. The values are in good agreement with those in Runs 1 to 3. It will be observed that the lengths, whether calculated for the rod or coil model, remain practically constant over that range of $r$ where the greatest viscosity drop occurs. This surprising result will be dealt with more fully in the discussion.

**Reversibility of the Reaction with Mercuric Chloride.**—To investigate the possibility that mercuric chloride might be reacting with SDNA to produce covalent as well as the expected ionic linkages with the singly charged –P–O$^-$ groups, an experiment (Run 5) was designed which would reverse the reaction if the linkages were even feebly ionized, as evidenced by a reversal to the original molecular weight and shape.

The angular intensity distribution of 25 ml. of buffer solution was first measured; 10 ml. of an

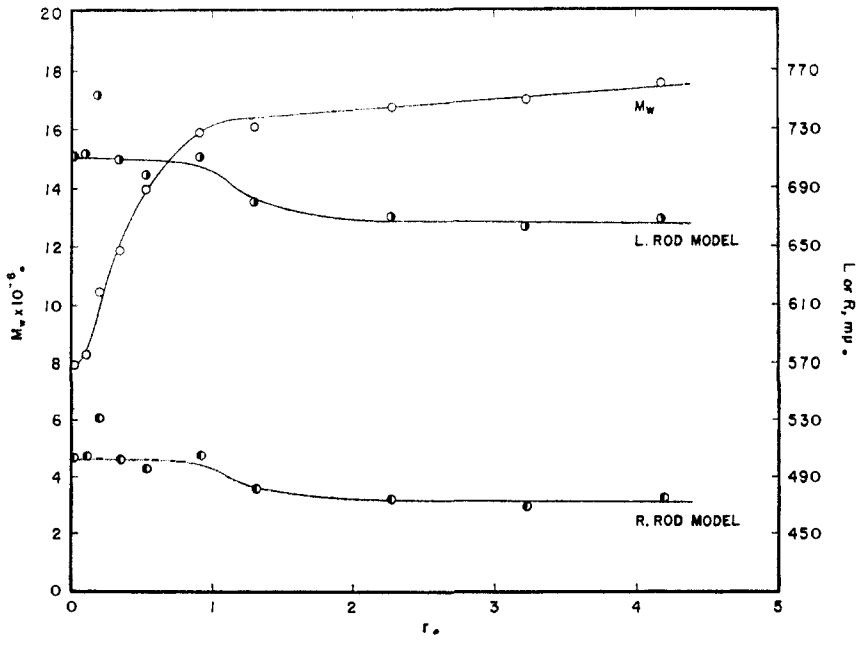


Fig. 9.—Molecular weights and average sizes of SDNA at various values of $r$. Sizes are estimated from rod and coil models; run 4.

(28) J. T. Edsall, H. Edelhoch, R. Lontie and P. R. Morrison, THIS JOURNAL, **72**, 4641 (1950).

SDNA solution in buffer was added and the angular intensity distribution was again measured. This procedure was also followed after addition of 0.60 ml. of mercuric chloride in buffer (making $r$ = 4.30) and a final addition of 0.60 ml. of potassium cyanide solution in buffer. The initial concentration of SDNA was $2.74 \times 10^{-4}$ g./ml. and the final concentration $2.67 \times 10^{-4}$, a negligible difference in view of the minor concentration dependence of $Kc/R_{\theta u}$. The final concentration of mercuric chloride was $3.53 \times 10^{-3}$ $M$ and that of potassium cyanide $14.4 \times 10^{-3}$ $M$, 4.06 times as great. Figure 10 shows the results obtained. It is clear that, in spite of the large change in molecular weight and size induced by the mercuric chloride, these changes can be completely reversed by cyanide which forms a very feebly ionized complex with mercuric ion

$$Hg^{++} + 4CN^- \rightleftharpoons Hg(CN)_4^=;\ K = 10^{-41}$$

This experiment makes the possibility of mercury–carbon bonds or irreversible changes resulting from oxidation–reduction extremely unlikely and supports the belief that the bonds between SDNA and $Hg^{II}$ are at least partially ionic in nature and presumably links between mercuric ion and P–O$^-$ groups.

**Electrophoresis Experiments.**—Electrophoresis studies were made in order to provide qualitative information on the state of charge of untreated SDNA as compared with that of SDNA treated with mercuric chloride in the region of large $r$. A Tiselius type of apparatus was employed equipped with the Philpot–Svensson cylindrical lens schlieren system. The $p$H was 6.9$_8$ and 6.7$_0$, the latter for the buffer containing mercuric chloride. The results may be seen in Table II.

TABLE II

ELECTROPHORESIS OF SDNA AT 2°

| Concn. SDNA, g./liter | Concn. mercuric chloride, g./liter | $r$ | Mobility cm.$^2$/sec./volt |
|---|---|---|---|
| 3.00 | 2.86 | 1.15 | 1.23 ± 0.04 |
| 3.00 | 0 | 0 | 1.23 ± 0.04 |

It is clear that there is no change in the mobilities within the experimental error, an unexpected result in view of the marked effect of the reagent on the other physical properties investigated.

**Binding Experiments.**—Equilibrium dialysis experiments were undertaken in order to correlate the data obtained from the physical measurements. A colorimetric method[29] employing dithizone was used for analysis of $Hg^{II}$. The absorption of a series of standards was measured at 485 mμ in a Beckman model B spectrophotometer yielding an extinction coefficient $(-\log I/I_0)/cd$ equal to 0.361 ml./γ/cm. in chloroform. The standard deviation from this mean of nine samples was ±0.004, Beer's law being obeyed with this precision up to the highest $Hg^{II}$ investigated, 5.0 γ/ml. Excess dithizone was removed with approximately 9 $N$, metal-free ammonium hydroxide and the mercury dithizonate color stabilized with acetic acid.[30] The solution of dithizone in chloroform was freed of oxidation products before use by shaking with 9 $N$ ammonium hydroxide and reacidification with redistilled, metal-free hydrochloric acid. This leaves the oxidation products in the aqueous phase.

In each binding experiment, 20 ml. of SDNA solution of concentration $9.7_8 \times 10^{-4}$ g./ml. was placed in dialysis bags (Visking casing) and immersed in 20 ml. of buffer containing varying amounts of mercuric chloride. The bag and external solution were contained in a glass test-tube with ground glass joints. The tubes were placed in a thermostat

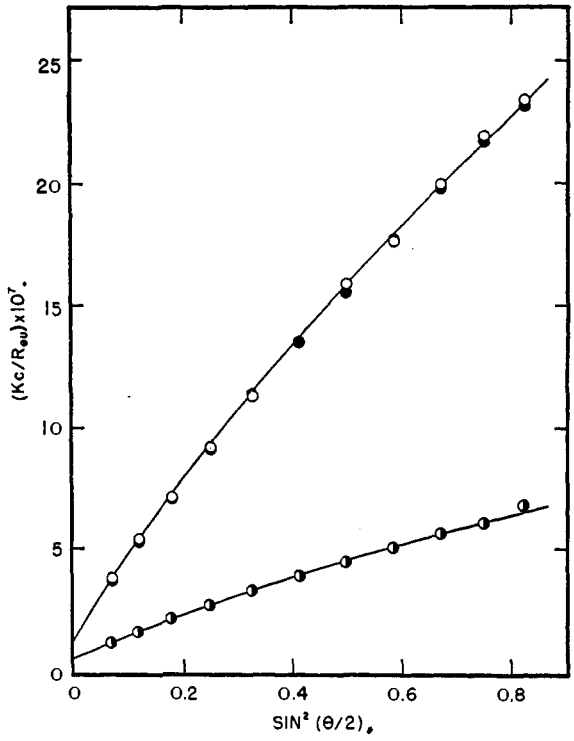


Fig. 10.—Zimm plot for reversibility experiment: open circles, SDNA in 0.101 $M$ citrate buffer; half shaded circles, SDNA in buffer with added mercuric chloride $r$ = 4.30; shaded circles, SDNA after addition of cyanide to buffer with added mercuric chloride.

at 25° for five days during which they were occasionally inverted and gently shaken manually. At the end of five days samples of the external solution were removed and aliquots analyzed for $Hg^{II}$. Table III and Fig. 11 give the results of this study. As Fig. 11 indicates, the binding experiment closely parallels the results of viscosity and light scattering measurements in that a large change in the slope of the curve appears in the same region of $r$ (compare with Figs. 1 and 9). The paucity of data and their lack of accuracy in the region of large $r$ unfortunately prevent analysis of the binding data in the manner of Klotz,[31] Scatchard[32] and others. They are most useful, however, in analysis of the light scattering data.

TABLE III

BINDING OF $Hg^{II}$ BY SDNA IN 0.101 $M$ CITRATE BUFFER

| Total mg. HgII without SDNA[a] | Total mg. HgII with SDNA | Mg. HgII bound by SDNA | Moles HgII bound per mole[b] nucleotide | $r$, total moles HgII per mole nucleotide inside membrane | Moles dimer per mole monomer | $R_i$, mμ |
|---|---|---|---|---|---|---|
| 0.717 | 0.064 | 0.653 | 0.0544 | 0.0571 | 0.00 | 714 |
| 2.22 | .166 | 2.06 | .172 | .179 | .078 | 542 |
| 3.85 | .648 | 3.20 | .268 | .294 | .18 | 475 |
| 6.68 | 2.52 | 4.16 | .346 | .452 | .32 | 435 |
| 9.69 | 5.24 | 4.45 | .371 | .589 | .46 | 416 |
| 14.14 | 9.12 | 5.02 | .419 | .798 | .61 | 408 |
| 21.38 | 17.16 | 4.22 | .352 | 1.07 | .64 | 392 |
| 30.94 | 22.52 | 8.42 | .702 | 1.64 | .57 | 385 |
| 47.67 | 40.4 | 7.27 | .604 | 2.28 | .51 | 387 |
| 117.4 | 107.2 | 10.2 | .852 | 5.32 | | |

[a] Corrected for binding of 0.722 mg. $Hg^{II}$ by membrane. [b] Based on average nucleotide weight of 326.

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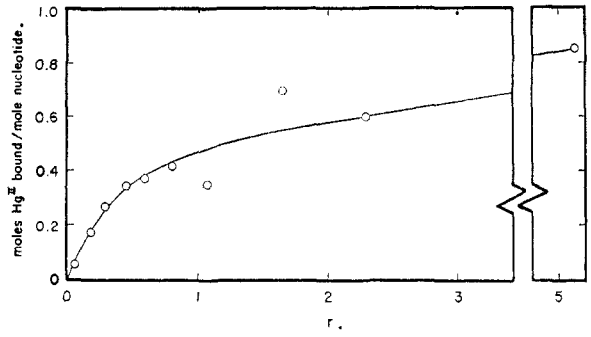


Fig. 11.—The number of moles of $Hg^{II}$ bound per mole nucleotide as a function of $r$ in 0.101 $M$ citrate buffer at 25°.

## Discussion

Examination of the data makes it clear that we are dealing not only with the binding of $Hg^{II}$ but also with the aggregation of SDNA. It is not possible to account for the increased molecular weight of the SDNA entirely in terms of the increment resulting from $Hg^{II}$ binding, even if it is assumed that $HgCl_2$ rather than $Hg^{++}$ is the species bound. For example, the molecular weight which would be calculated from the binding of 0.60 mole of $Hg^{II}$ per mole nucleotide as mercuric chloride at $r = 2.28$ (*cf.* Table III) would be only $12.1 \times 10^6$ as compared with the observed value of $16.7 \times 10^6$. Some aggregation, therefore, must occur in addition to binding.

In order to estimate the extent of aggregation we may assume that only monomers and dimers exist in solution and that the number of moles of $Hg^{II}$ bound per mole nucleotide is the same for both. By a monomer is meant a molecule of molecular weight $8.1 \times 10^6$ plus the molecular weight of the attached $Hg^{II}$, assumed to be mercuric ion. The ratio of dimer to monomer is then easily calculated making use of the fact that light scattering measurements yield a weight average molecular weight.[33] The sixth column of Table III gives the ratio of dimer to monomer calculated this way. In the region of high $r$ one needs approximately one dimer to two monomers in order to explain the observed molecular weight. No significance is attached to the apparent maximum in the dimer–monomer ratio because of the assumption involved in the calculation.

To estimate the size of the monomers at various values of $r$ one needs only the additional assumption that $R_2$, the root mean square distance between the ends of the dimer, is twice that of the monomer. Just as in the case of rod-like particles,[21] it is easily demonstrated that the square of the average RMS distance obtained experimentally is the $Z$ average of the squares of the RMS distances of the individual species.

This enables calculation of $R_1$. The seventh column of Table III was calculated this way using smoothed values of $R$ from Fig. 9. The monomer steadily decreases in size with increasing $r$ to nearly half the size of the unreacted SDNA molecule. It should be noticed that it is unnecessary to make any assumptions to show that the over-all molecular size of SDNA has decreased, since this conclusion follows from the fact that although the molecular weight increases, the average dimensions remain constant or decrease (Fig. 9).

We may now examine the apparent anomaly mentioned earlier: that the *average* molecular dimensions, whether calculated for rod or coil, remain constant over that range of $r$ in which the greatest viscosity drop occurs.

Since SDNA is a polyelectrolyte it might be supposed that the viscosity decrease is attributable in a simple way to the ionic strength contribution of the mercuric chloride. This is not a very attractive idea, however, because mercuric chloride is largely un-ionized in solution, its ionization constant[34] being $1.6 \times 10^{-13}$. This means, for example, that at $r = 4.9$ in Fig. 1 the mercuric chloride, even if none were bound, could contribute only $1.8 \times 10^{-5}$ mole/liter to the ionic strength, which is negligible compared to the 0.60 mole/liter contributed by the buffer. The last column of Table III suggests that there are three principal factors involved in the viscosity decrease, two operating in its favor and one in opposition to it. If we regard the monomer and dimer as roughly coil-like,[35] then there would be a decrease in viscosity arising from the decrease in $R_1$. At low values of $r$, where the amount of dimer is small, this is probably the most important factor. At high values of $r$, the dimer is very little larger than the unreacted SDNA molecule and since there is about one dimer to two monomers an additional contribution to the decrease in viscosity is to be expected from the reduction in the concentration of hydrodynamic units. In the intermediate region of $r$ the dimer is larger than the unreacted SDNA molecule and would be expected to make a positive contribution to the viscosity of the solution. Evidently, this factor is everywhere outweighed by the others.

The decrease in over-all size of the SDNA molecule is in itself interesting because it is the first demonstration that such a reduction in the size of this molecule can be obtained by a salt and reversed. The shrinkage may arise in two ways. The electrostatic repulsions between charged phosphoryl groups, which tend to stretch the molecule, are greatly diminished by binding of $Hg^{II}$, permitting the molecule to contract. This behavior would resemble that of a weak polyelectrolyte such as polyacrylic acid, to which ordinary salt is added. In addition, the mercury may also form some intramolecular links of the type P–O–Hg–O–P, which would hold the molecule together in a more condensed form.

The interpretation of the electrophoresis experiments can be made consistent with the previous conclusions. In the experiment conducted at $r = 1.15$ there are, by graphical interpolation, 0.47 mole of $Hg^{II}$ bound per nucleotide or approximately one $Hg^{II}$ to every two nucleotides. The monomer is about half the size of the original SDNA molecule while the dimer is approximately the same size and

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(35) The succeeding argument is independent of a choice between rod-like and coil-like molecules, since the viscosity contribution of the monomers would decrease if either $R_1$ or $L_1$ decreased.

shape. Half the original charge of the dimer is lost since one $Hg^{II}$ is bound to every two nucleotides. This means that the dimer has not only about the same size and shape as the unreacted SDNA molecule, but also the same charge and hence the same mobility. The loss of charge in the monomer on the other hand, is apparently compensated by its reduction in size.

There remains one further point of interest. The aggregation of SDNA by mercuric chloride does not continue into precipitation such as occurs, for example, in the case of polyacrylic acid[36] to which increasing amounts of barium, strontium or calcium ion are added. The most likely explanation appears to be that one is dealing with a competition for mercuric ion by phosphate groups on the SDNA and chloride ion itself. Mercuric chloride is a poor electrolyte but mercuric nitrate, which is a strong electrolyte, is capable of providing sufficient mercuric ion to precipitate SDNA from solution. Mercuric acetate, a half-strong electrolyte in the sense used by Sidgwick[37] also furnishes more mercuric ion than the chloride and is also capable of precipitating SDNA.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

# Mycomycin. I. Isolation, Crystallization and Chemical Characterization

BY WALTER D. CELMER AND I. A. SOLOMONS

Mycomycin, a previously described unstable antibiotic, has been isolated in crystalline form and characterized as a highly unsaturated, optically active, straight-chain carboxylic acid having the empirical formula $C_{13}H_{10}O_2$. Thermal instability and sensitivity to oxygen are characteristic of the pure compound. Infrared absorption data suggest that the structure of mycomycin contains acetylenic and allenic groups. The methyl ester has been prepared and the reduction product of mycomycin has been identified as *n*-tridecanoic acid.

Mycomycin is an unstable antibiotic isolated by Johnson and Burdon[1] from the elaboration products of *Norcardia acidophilus*. It is active *in vitro* against numerous microörganisms, including streptomycin sensitive and resistant strains of *Mycobacterium tuberculosis* and a variety of pathogenic fungi.[2] A unit of activity has been defined as "the least amount which will inhibit the growth of *Bacillus mesentericus* in tryptose phosphate broth for 16 hours at 37°."[2]

In the original isolation method[3] the broth was filtered, acidified and extracted with hexane. The antibiotic was then re-extracted from the hexane solution into sodium phosphate buffer. The aqueous concentrates exhibited absorption maxima at 267 and 281 mμ which could be correlated with the microbiological activity. Extreme sensitivity to heat was evidenced by rapid loss of both ultraviolet absorption and microbiological activity unless the concentrates were stored at Dry Ice temperatures.

In order to facilitate further studies on the biological and chemical properties of mycomycin, it seemed important to improve the method of isolation and to seek some means of stabilizing the antibiotic. Preliminary observations indicated that the stability of mycomycin was greatly enhanced by working with relatively dilute solutions at low temperatures in an inert atmosphere. In an eight-plate countercurrent distribution of a dilute solution of the antibiotic between chloroform and cold 2% *p*H 7.0 phosphate buffer, the experimental curve showed little divergence from a calculated curve (Fig. 1). The antibiotic was finally obtained as white, crystalline needles. Repeated low temperature crystallizations from various solvents did not alter the characteristic ultraviolet spectrum of the material. The crystals exploded at about 75° in a capillary tube sealed with nitrogen. Though enhanced stability was observed with crystalline mycomycin, complete retention of activity is possible only by storage at a temperature of −40° or lower.

Mycomycin is an acid having a neutral equivalent of about 200 as determined by titration in aqueous methanol. It was found to contain carbon, hydrogen and oxygen, corresponding to the empirical formula $C_{13}H_{10}O_2$. Mycomycin is levorotatory with a specific rotation $[\alpha]^{25}D$ −130 in absolute ethanol Molecular extinction coefficients in methanol at 281 and 267 mμ are 67,000 and 61,000, respectively (see Fig. 2). The infrared absorption spectrum is shown in Fig. 3. The microbiological potency of the crystalline compound is about 50,000 U./mg. Attempts to prepare crystalline salts were unsuccessful due to decomposition.

When the crystals are stored at 27° in a nitrogen atmosphere or *in vacuo*, the half-life is only three

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