was crystallized from alcohol–water to give a material which melted at 247–248°.

Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: N, 19.45; S, 11.10. Found: N, 19.28; S, 11.21.

TABLE I

ACETYLSULFANILAMIDOINDAZOLES

Acetylsulfanilamidoindazole	M. p., °C. (uncor.)	Formula	N Analyses, %[6] Calcd.	Found
3-	253–255	$C_{15}H_{14}N_4O_3S$	16.97	17.11
5-	250–252	$C_{15}H_{14}N_4O_3S$	16.97	16.60
6-	245–246	$C_{15}H_{14}N_4O_3S$	16.97	16.72
7-	258–260	$C_{15}H_{14}N_4O_3S$	16.97	16.84

TABLE II

SULFANILAMIDOINDAZOLES

Sulfanilamidoindazole	M. p., °C. (uncor.)	Formula	N Analyses, % Calcd.	Found
3-	225–226	$C_{13}H_{12}N_4O_2S$	19.45	19.61
5-	247–248	$C_{13}H_{12}N_4O_2S$	19.45	19.28
6-	195–196	$C_{13}H_{12}N_4O_2S$	19.45	19.40
7-	254–256	$C_{13}H_{12}N_4O_2S$	19.45	19.64

Preparation of 3-Sulfanilamidoindazole.—A solution of 23 g. (0.07 mole) of 3-acetylsulfanilamidoindazole in 250 ml. of 20% alcoholic hydrogen chloride was shaken at room temperature for fifteen hours. During this time, the hydrochloride of 3-sulfanilamidoindazole separated; it was filtered off, dissolved in water and the aqueous solution neutralized in the cold to precipitate the crude product. This crude material was then crystallized from alcohol or from 50% acetic acid, to yield 11.5 g. (57.3%) of 3-sulfanilamidoindazole of melting point 225–226°.

Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: N, 19.45. Found: N, 19.61.

Summary

The synthesis of a series of acetylsulfanilamidoindazoles and sulfanilamidoindazoles has been described. *In vitro* tests indicate that the sulfanilamidoindazoles have a marked bacteriostatic and, in some cases, bactericidal action. Several of these compounds show promising activity against streptococcus hemolyticus and pneumococcus infections in mice.

RENSSELAER, NEW YORK

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(6) The authors are indebted to Miss E. A. Bass and Miss H. M. Hutchinson for the microanalyses.

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Effect of *p*H Changes upon Some of the Properties of Sodium Thymonucleate Solutions[1]

BY CHARLES F. VILBRANDT[2] AND HOWARD G. TENNENT[3]

Introduction

The molecular sizes and shapes of several nucleic acids, calculated from their sedimentation and diffusion behavior, have been reported in a previous article.[4] The molecular cross-sectional diameters of the different nucleic acid preparations studied were calculated to be approximately the same, about 14 Å., whereas the molecular lengths varied from 40 to 5000 Å.

It seemed significant that the longest nucleic acid molecules were those which had been prepared by Hammarsten's method[5] in which only solutions neutral to litmus and temperatures close to 0° are used. The samples prepared by more vigorous methods had much lower weights. These differences suggested that the smaller nucleic acid molecules were fragments of long chains which had been degraded in the process of isolating them from their natural sources. A study of the effects produced on a nucleic acid of high molecular weight by *p*H changes from neutrality to acid and alkaline conditions has been made to test this hypothesis.

A few examples of the effect of acids and bases on the physical properties of nucleic acid solutions have been reported in the literature. Jones and Austrian[6] and Jones,[7] using the "α-salt" of thymonucleic acid prepared by Neumann's method,[8] found that the viscosity of a 4% solution was decreased by the addition of either acetic acid or sodium hydroxide. The solution could be changed back and forth from a gelatinous to a fluid state by the alternate addition of acid and alkali. The optical rotation was decreased by the addition of either reagent. Hammarsten,[5] using solutions of sodium thymonucleate prepared by his method,

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(1) More complete details of this work are to be found in theses of the authors submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree in June, 1942.

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

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

(6) W. Jones and C. R. Austrian, *J. Biol. Chem.*, **3**, 1 (1907).

(7) W. Jones, *ibid.*, **5**, 1 (1908).

(8) A. Neumann, *Arch. f.* (*Anat. u.*) *Physiol.*, **22**, 374 (1898).

reported a decrease in their relative viscosity on addition of hydrochloric acid but did not describe any experiments involving neutralization of the acidified solution. He found also that a 1% solution of his sodium thymonucleate, heated with a small amount of sodium hydroxide on a water-bath for one minute, would solidify to a gel when cooled and neutralized with hydrochloric acid. He believed that the salt produced by this treatment was identical with the "α-salt" of Neumann.

In this report we describe the effect of *p*H changes on the viscosity and on the sedimentation and diffusion behavior of a solution of sodium thymonucleate. The sample of thymonucleate used was chosen because of its high molecular weight and relatively narrow distribution of weights, which made it a good starting material for a study of polymer degradation.

Experimental

The experiments were divided into four parts. All of them were made with a solution which contained 0.3% sodium thymonucleate[9] and 1% sodium chloride. First, the *p*H of the original solution (5.6) was lowered in several steps to 2.6 by the addition of hydrochloric acid, and the viscosity of the solution was measured at each step. If there was a noticeable change in viscosity with time after the hydrochloric acid was added, more measurements were made until it became constant. Second, the same procedure was followed with another portion of the same solution except that the *p*H was raised stepwise to 11.6 by the addition of sodium hydroxide. Third, one portion of the original solution was adjusted directly to *p*H 2.6 and another to *p*H 11.6. Their viscosities dropped immediately to values very little above that of the solvent. The solutions were then returned to neutrality and viscosity readings were taken at suitable intervals until they approached constant values. Fourth, sedimentation velocity measurements were made on the original solution, the solution at *p*H 2.6, and the solutions which had been adjusted to the acid and alkaline extremes and neutralized again. All sedimentation velocity determinations were made after the solution viscosities had reached their final values. Two diffusion experiments were performed with a solution at *p*H 2.6.

Viscosities were measured with an Ostwald type of capillary viscometer of 12-cc. capacity in a water-bath at 25.07 ± 0.02°. The densities of the 1% sodium chloride solution which was used as solvent and of the sodium thymonucleate solution, necessary for the calculation of relative viscosities, were obtained in the course of partial specific volume determinations previously described.[4] It was assumed that the slight dilutions due to the addition of acid and base during the *p*H adjustments did not affect this density. The *p*H measurements were made with a Coleman style 200 *p*H electrometer calibrated against buffers of *p*H 4.0 and 9.2. The hydrochloric acid and sodium hydroxide solutions were added from a microburet of 0.2-cc. capacity graduated to 0.01 cc., and their normalities were chosen so that the desired *p*H changes could be made with no appreciable dilution of the sodium thymonucleate solutions.

The methods of measuring sedimentation and diffusion behavior already have been described.[4]

Results

The final relative viscosities observed after the successive *p*H adjustments to values above and below that of the original solution are shown in Fig. 1. No measurements were made below *p*H 2.6 because the nucleic acid precipitated beyond that point. The irregularity in the curve at *p*H 5.6 resulted from a drop in the relative viscosity of the untreated original solution during the period between the experiments with the acid solutions and those with the alkaline preparations.[10] After each adjustment to a *p*H above or below that of the original solution, the corresponding de-

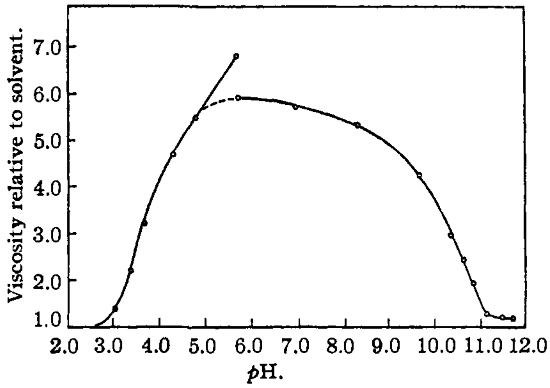

Fig. 1.—Dependence of relative viscosity of 0.3% STN-2 (in 1% NaCl solution) on *p*H.

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[9] The sodium thymonucleate used (STN-2) was described in a previous article.[4]

[10] Hammarsten[8] found the same decrease in relative viscosity with time for freshly prepared sodium thymonucleate solutions and showed that it occurred even with carbon dioxide-free sterile solutions in quartz vessels.

crease in viscosity to a new equilibrium value was extremely rapid. As a result, measurements of the rate of viscosity decrease were not possible.

The increase in relative viscosity of the 0.3% STN solutions in 1% NaCl after neutralization from *p*H 2.6 and 11.6 is shown in Fig. 2. The rate of increase of relative viscosity after neutralization of the solutions was readily measurable. However, the final values of viscosity which were observed were only slightly more than half those of the original solution.

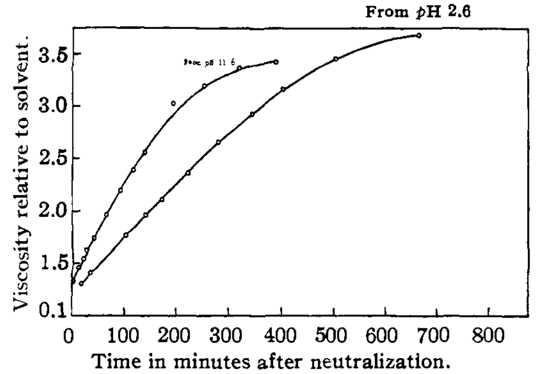

Fig. 2.—Increase in relative viscosity of 0.3% STN-2 in 1% NaCl solution after neutralization.

In Fig. 3 are shown the results of diffusion experiments made on a sodium thymonucleate solution at *p*H 2.6. Charlier's equation for the transformation of the experimental diffusion curves to normal coördinates was used to compare these curves with those which would have been obtained if the solute molecules were all identical in size and weight. The method of transformation and the significance of the coördinates used are described by Lamm.[11] The open and shaded circles in Fig. 3 represent the normalized results of two experiments and the solid curve shows the behavior which would be exhibited if the solute molecules were all identical. The large difference between experimental points and the theoretical curve indicates that the sodium thymonucleate which had diffused was highly polymolecular. Similar diffusion experiments on the original solution before it was acidified to *p*H 2.6 yielded points which fell on the theoretical curve for a homogeneous solute within the limits of experimental error.

The behavior of the thymonucleate solution at *p*H 2.6 in the Svedberg high velocity, oil-turbine ultracentrifuge was again characteristic of that of a polydisperse system. The sedimentation diagrams were asymmetric and no accurate sedimentation constants could be obtained from them because of their rapid spread. The results of sedimentation velocity experiments with the thymonucleate solutions which had been neutralized from *p*H values of 2.6 and 11.6 were very much alike. The sedimentation diagrams were asymmetric and very sharp during the entire course of the sedimentation experiments. The sedimentation velocity constants calculated from these diagrams decreased with successive time intervals during each experiment. The minimum sedimentation constant for the largest sedimenting units was estimated to be about 150 *S*.

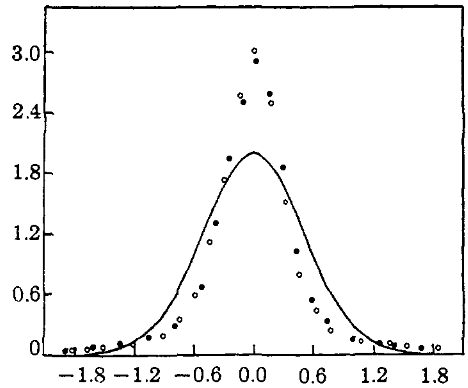

Fig. 3.—Normalized diffusion curve for STN-2 at *p*H 2.6. Solid line is normal curve for a homogeneous substance. Points show results of two experiments. (Scale line displacement *vs.* distance in cell.)

Discussion

The following description of the effect of *p*H on the sodium thymonucleate molecules is advanced to explain the changes in physical properties which were observed. In the original untreated solution, these molecules were present as long chains of approximately the same length. On addition of either acid or alkali, the chains were cut in a random fashion at various points along their lengths to give a wide distribution of molecular weights whose average was much lower than the original value. Neutralization of the acid and alkaline solutions caused a recoupling of the chain fragments, again in a random manner, to produce a very wide distribution in chain length ranging from the small fragments present before neutralization to chains much longer than those present in the original solution.

The process of severing the chain molecules which results when the original thymonucleate

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[11] O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, **4**, No. 6, 10 (1937).

solution is made acid or alkaline could occur simultaneously at many points along their length, and the corresponding decrease in solution viscosity would be rapid. On the other hand, recoupling of the chain fragments after neutralization of the solution would be dependent upon a suitable orientation (end to end) of the reacting units, and therefore probably would be a slow process compared to the previous degradation of the chain molecules. The rise in solution viscosity would be correspondingly slow.

The behavior of the neutralized solutions in the ultracentrifuge can be explained by the presence of the very long molecules formed through repolymerization. Although not originally present in very large numbers, these long molecules would be concentrated by sedimentation. They would then tend to bind one another into a network with some of the properties of a gel, which would produce the artificial sharpening of sedimentation diagrams and progressively decreasing sedimentation rates which were observed.

The sensitivity of the sodium thymonucleate molecules to *p*H changes from neutrality, even at room temperature, emphasizes the necessity for extreme care in the extraction of nucleic acids from their natural sources. Methods of isolation which involve the use of acids or bases, particularly at elevated temperatures, cannot be expected to yield products whose physical properties are useful guides to the nature of nucleic acids as they exist in the living cell. This point has been made before by others, but it cannot be too strongly emphasized, since some confusion exists in the literature because it has not always been considered.

Acknowledgment.—We wish to express our gratitude to the Wisconsin Alumni Research Foundation and to the University Research Committee for the financial assistance which has made these studies possible. We also wish to thank Dr. J. W. Williams for his counsel and help throughout this work.

Summary

Measurements have been made of the effect of *p*H changes on the viscosities of solutions of a high molecular weight sodium thymonucleate. The data suggest that this salt acts in neutral solution as a linear high polymer which is rapidly depolymerized to a considerable extent when the *p*H is changed to 2.6 or to 11.6. The effect is reversed when the acid and alkaline solutions are neutralized. The material then repolymerizes slowly. Sedimentation velocity and diffusion experiments show that both the degraded and the repolymerized nucleic acids have a wider distribution of molecular weights than the original substance and that some of the molecules in the repolymerized portion are much larger than those which were present in the original solution.

MADISON, WISCONSIN RECEIVED JUNE 11, 1943

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Catalytic Dehydrogenation. I. Catalytic Conversion of Alcohols into Aldehydes, Paraffins and Olefins

BY ELMER J. BADIN

The liquid-phase nickel-catalyzed conversion of representative members of the homologous series of primary aliphatic alcohols into the corresponding aldehyde, an unsaturated hydrocarbon of one less carbon atom and the saturated hydrocarbon of one less carbon atom has been studied. Although catalytic dehydrogenation to the aldehyde has been carried out by various investigators using different catalysts, especially in the vapor phase, further "deformylation" has not been completely studied. By deformylation is understood the splitting out of carbon monoxide and hydrogen from the aldehyde.

In view of the fact that hydrocarbons with an odd number of carbon atoms and higher paraffins containing more than eight carbon atoms are difficult to obtain or prepare by the usual chemical methods, catalytic dehydrogenation followed by deformylation and hydrogenation, occurring in one step without the use of pressure or an external source of hydrogen, affords an easy method of preparing these hydrocarbons. The reaction may be regarded as a preparative one for aldehydes and hydrocarbons.

The catalyst used in this series of experiments was Raney nickel and the reaction was carried out