Tubes 59–75 contained 19.8 mg. (11% of the crude estriol fraction) of estradiol-17β based on ultraviolet absorption spectrum. This product undoubtedly arose from the lithium aluminum hydride reduction of either estrone acetate, which contaminated the once recrystallized estrone enol diacetate employed in the reactions, or derived from the enol diacetate which was not oxidized with perbenzoic acid.

B. By Reduction of the Epoxide X.—Estrone enol diacetate ($\Delta^{1,3,5,16}$-estratetraene-3,17-diol diacetate (IX)) prepared from 5.0 g. of estrone was poured through a short alumina column and recrystallized four times from petroleum ether. The enol diacetate thus prepared (3.4 g., m.p. 145–149°) was oxidized at room temperature overnight with 3.4 g. of perbenzoic acid in 80 ml. of benzene to give 3.2 g. of epoxyacetate (16α,17α-epoxy-$\Delta^{1,2,5}$-estratriene-3,17β-diol diacetate (X)). The crude epoxide acetate was directly reduced with 3 g. of lithium aluminum hydride in benzene and ether to give 2.40 g. of crude estriol.

A portion (200 mg.) of the crude estriol was purified by countercurrent distribution as above. The individual tubes containing estriol were combined and 124 mg. of product, m.p. 278.5–284°, ϵ_{2800} = 2090 (ethanol), was obtained. Estriol thus obtained represents 62% of the crude estriol fraction or an over-all yield from estrone enol acetate of 54%.

There was 57 mg. of the unknown compound which had a distribution coefficient intermediate between estriol and estradiol. Only 10 mg. of estradiol was obtained, indicating that the starting material, estrone enol diacetate, was relatively free of estrone acetate.

Acknowledgments.—We are indebted to Dr. Charles T. Beer and Millicent Houde for the countercurrent distribution of estriol and to Milton Heffler for technical assistance. We wish to express our appreciation to Friederike Herling for the determination and interpretation of the infrared spectra.

NEW YORK, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENTS OF PEDIATRICS AND BIOCHEMISTRY, UNIVERSITY OF CHICAGO, AND LARABIDA JACKSON PARK SANITARIUM]

Testicular Hyaluronidase in Relation to Micelle Formation by Inactivating Agents[1]

BY MARTIN B. MATHEWS

RECEIVED DECEMBER 7, 1953

The inactivation of testicular hyaluronidase by an homologous series of sulfated aliphatic alcohols has been studied. The concentrations at which the detergents produced equal rates of inactivation was determined and found to correlate well with the critical micelle concentrations of the detergents. The inactivating effect of the detergents, which increased with time, was not reversed by dilution or precipitating agents. This behavior was not found for polyanionic compounds like heparin, which has been presumed to inhibit competitively on the basis of structural similarity to the substrate. The action of other compounds (*d*-α-tocopheryl phosphate and hexylresorcinol) was found to resemble that of the detergents, and may be similarly interpreted on the basis of the micelle-forming properties possessed by molecules with a strongly hydrophilic group and a long hydrocarbon chain. It is proposed that either anionic micelles interact with hyaluronidase or that a similar micellar aggregate forms at the enzyme surface. A high affinity for the enzyme might be predicted for such an aggregate since it resembles the natural substrates with respect to size, shape and high negative charge. In accordance with this view is the observation that the known organic inhibitors of hyaluronidase which are effective at very low concentration are all negatively charged in solution and either are of high molecular weight or else are very likely associative colloids.

Introduction

The *in vitro* activity of testicular hyaluronidase is reduced in the presence of low concentrations of a large number of "natural" and synthetic inhibitors.[2–11] Chondroitinsulfuric acid, a natural substrate of the enzyme, possesses in solution a configuration and charge[12,13] similar to that of anionic detergent micelles.[14] The experiments to be described below were undertaken in order to investigate the hypothesis that the inhibition of hyaluronidase by low molecular weight organic compounds is related to the micelle-forming properties of these compounds.

Experimental

Materials.—The highly purified sodium salts of straight chain sulfated fatty alcohols with, respectively, 8, 10, 12, 14 and 16 carbon atoms were obtained from M. L. Corrin of the Department of Chemistry, The University of Chicago. Sodium di-(2-ethylhexyl)-sulfosuccinate (aerosol OT) was purchased as a pure solid (99% purity claimed by American Cyanamid Corp.). A heparin preparation (lot no. 199) was obtained from Hynson, Westcott and Dunning, Inc. An ammonium salt of aurin tricarboxylic acid (ATA) was a specially purified (Eastman) preparation obtained from J. Schubert of Argonne National Laboratories. The *d*-α-tocopheryl phosphate (93% purity) was a gift by S. R. Ames of Distillation Products, Inc. Crystalline hexylresorcinol (m.p. 69°) was generously supplied by Winthrop-Stearns Co. The partially purified hyaluronidase[15] (bull testes) preparations as well as a hyaluronic acid[16] (human umbilical cord) preparation have been described.

Methods.—The turbidimetric assay[16] for hyaluronidase activity was used with substitution of the buffer employed for dilution of the enzyme. The method consisted essentially of the following steps: (1) incubation of 1 cc. of a known dilution of enzyme in 0.01 *M* phosphate buffer *p*H 7.0 and 0.45% sodium chloride with 1 cc. of hyaluronic acid in 0.3 *M* phosphate buffer *p*H 5.5 at 38° for 45 minutes; (2) addition of 10 cc. of acidified bovine serum albumin (Armour fraction V) solution to produce a turbidity; (3) measurement of the optical density, which is quantitatively related to the amount of hyaluronic acid remaining unde-

(1) (a) Presented before the Division of Biological Chemistry at the Chicago Meeting of the A.C.S., September, 1953; (b) supported by grants from the Helen Hay Whitney Foundation, the Chicago Heart Association and the Variety Club of Illinois; (c) work done during tenure of a Research Fellowship of the American Heart Association.

(2) H. Gibian, *Angew. Chem.*, **63**, 105 (1951).

(3) D. McClean, *J. Path. Bacteriology*, **54**, 284 (1942).

(4) T. Astrup and N. Alkjaersig, *Nature*, **166**, 568 (1950).

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(6) Z. Hadidian and N. W. Pirie, *Biochem. J.*, **42**, 266 (1948).

(7) L. Hahn, *Nature*, **170**, 282 (1952); L. Hahn and J. Fekete, *Acta Chem. Scand.*, **7**, 798 (1953).

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(9) W. H. Miller and A. M. Dessert, *Ann. N. Y. Acad. Sci.*, **52**, 167 (1949).

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(11) B. Calesnick and R. Beutner, *Proc. Soc. Exp. Biol. Med.*, **72**, 629 (1949).

(12) M. B. Mathews, *Arch. Biochem. Biophys.*, **43**, 181 (1953).

(13) A. Levine and M. Schubert, THIS JOURNAL, **74**, 5702 (1952).

(14) W. Philippoff, *Disc. Faraday Soc.*, No. 11 (1951).

(15) M. E. Freeman, P. Anderson, M. E. Webster and A. Dorfman *J. Biol. Chem.*, **186**, 201 (1950).

(16) A. Dorfman and M. L. Ott, *ibid.*, **172**, 367 (1948).

graded by the enzyme; (4) comparison of the behavior of the unknown enzyme solution with that of a standard enzyme solution to obtain a value of enzyme activity for the unknown solution in relative and arbitrary units.

The effect of various substances upon the activity of hyaluronidase was determined in the following manner. Eight units of enzyme activity in $^1/_2$ cc. of solution containing 0.45% sodium chloride and 0.02 *M* phosphate buffer *p*H 7.0 were mixed with $^1/_2$ cc. of 0.45% sodium chloride solution containing a definite concentration of the substance to be tested. The tube containing the mixture was placed in a bath at 38° for approximately 5 minutes, 1 cc. of hyaluronic acid solution added, and further incubated for 45 minutes. The assay was continued as described above. It was possible with this method to measure reliably the degree of inhibition in the range of 10 to 90%. Experiments were carried out with enzyme containing 130 units per mg. of protein and with more purified enzyme containing approximately 4000 units per mg. of protein. While this method yields results suitable for comparison with previously published data, it cannot be used to establish the nature of the inhibition.

For this purpose kinetic experiments were conducted by incubating 800 units of enzyme (0.2 mg. of protein) and added inhibitor in 0.45% sodium chloride solution with 0.01 *M* phosphate buffer *p*H 7.0 at 38°. Samples were withdrawn at fixed intervals of time, diluted 100-fold in a 0.45% sodium chloride solution with 0.01 *M* phosphate buffer *p*H 7.0 and 1 cc. of the diluted solution assayed for enzyme activity as described above.

The critical micelle concentration (CMC) of a compound was determined visually by the titration technique.[17] Pinacyanol chloride at 1×10^{-5} *M* was used with ionic concentrations, *p*H and temperature the same as those used in the final incubation period of the hyaluronidase procedure. Due to the low solubilities of some compounds, a few measurements were made at temperatures near 60°.

Results

Inactivation by Detergents.—The effect of various concentrations of detergent upon hyaluronidase activity in the turbidity assay procedure for a series of straight chain sulfated fatty alcohols are shown in Fig. 1, in which the concentration range (vertical line) corresponds to a degree of inactivation between 10 and 90%. The dotted line refers to sodium di-(2-ethylhexyl)-sulfosuccinate which was located at an arbitrary value of the abscissa. At concentrations higher than those indicated by the vertical lines the degree of inactivation was indistinguishable from 100%. At lower concentrations the degree of inactivation was essentially zero. Essentially similar results were obtained with enzyme preparations of different purity, *i.e.*, with different amounts of total protein present. The data show a general relationship between effective concentration range for inactivation and critical micelle concentration for the series of detergents.[18]

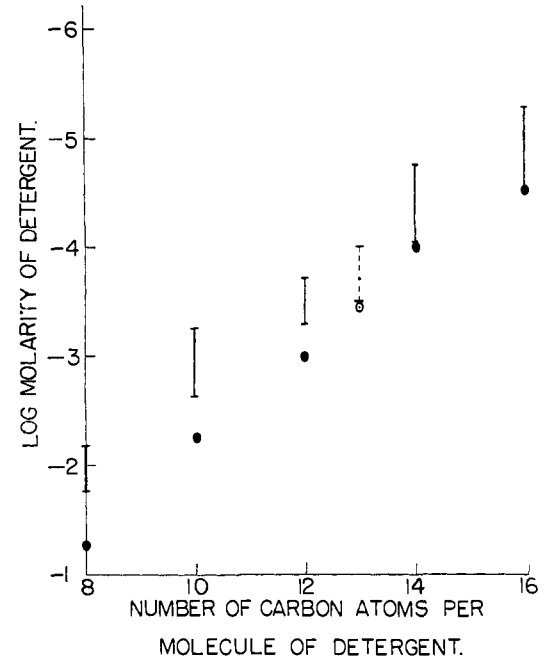


Fig. 1.—Inactivation of hyaluronidase by detergents in turbidity assay procedure; vertical lines are concentration ranges corresponding to degree of inactivation between 10% and 90%; ● indicates critical micelle concentration. Data are for straight chain sulfated fatty alcohols except for sodium di-(2-ethylhexyl)-sulfosuccinate (dotted line) which was arbitrarily positioned. Enzyme contained 130 turbidity reducing units per mg.

Time-Dependence of Inactivation.—The rate of degradation of hyaluronic acid was followed by withdrawing samples at 5-minute intervals up to the total period of 45 minutes incubation used in the turbidity assay for hyaluronidase. The data shown in Fig. 2 indicate an essentially linear relation between log optical density (*i.e.*, amount of

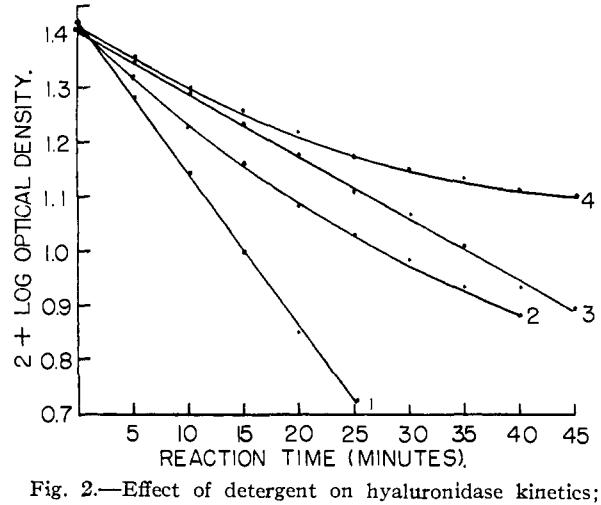


Fig. 2.—Effect of detergent on hyaluronidase kinetics; variation of relative amount of substrate remaining (optical density) with reaction time. Curves 1 and 2, respectively, 8 units of enzyme per cc. without and with 0.0003 *M* sodium dodecyl sulfate; curves 3 and 4, respectively, 4 units of enzyme per cc. without and with 0.0003 *M* sodium dodecyl sulfate. Enzyme contained 130 units per mg.

(17) M. L. Corrin, H. B. Klevens and W. D. Harkins, *J. Chem. Phys.*, **14**, 480 (1946).

(18) H. B. Klevens (*J. Phys. Colloid Chem.*, **52**, 130 (1948)) found that CMC data for an homologous series of straight chain surface active agents dissolved in pure water could be represented by the equation: log CMC = $A - BN$, where N is the number of carbon atoms in the molecule and A and B are empirical constants. Values of A varied considerably (1.42 to 2.03) depending upon the nature of the ionizing group in the molecule; values of B were approximately constant (0.290 to 0.296). For the homologous series of sulfated straight-chain fatty alcohols a recent report (H. B. Klevens, *J. Am. Oil Chemists Soc.*, **30**, 74 (1953)) gave A as 1.42 and B as 0.295. The CMC data on sulfated alcohols which appear in Fig. 1, although obtained in solutions of high ionic concentration, adequately fit the Klevens relation with $A = 2.17$ and $B = 0.43$. The increased value of the constant B is in agreement with the finding (M. L. Corrin and W. D. Harkins, THIS JOURNAL, **69**, 683 (1947)) that the extent of depression of the CMC brought about by the addition of equal amounts of salt to pure water solutions of surface active agents is greater the lower the value of the CMC in pure water.

TABLE I

INACTIVATION OF HYALURONIDASE BY VARIOUS COMPOUNDS

Compound	Type	Concn. for 50% inactivation in assay, *M*	CMC, *M*	Loss of enzyme upon prior incubation, %	Reversal of inactivation upon dilution, %
Sodium dodecyl sulfate	$ROSO_3Na$	3×10^{-4}	1×10^{-3}	100	0
Aurin tricarboxylic acid	$R(COONa)_3$	5×10^{-5}	.....	0	100
Heparin[a]	$[R(OSO_3Na)_5]$	2×10^{-4}	.....	0	100
d-α-Tocopheryl phosphate[b]	$ROPO_3Na_2$	5×10^{-5}	.....	100	100
Hexylresorcinol[c]	$R(OH)_2$	7×10^{-4}	1×10^{-3}	100	0

[a] Actual concentration for 50% inactivation was 0.25 mg./cc. The mole weight of the repeating unit containing 5 ester sulfate groups was taken as 1228 to obtain a *base molarity* for comparison purposes (M. L. Wolfrom, *et al.*, THIS JOURNAL, **72**, 5796 (1950)). [b] A CMC for *d*-α-tocopheryl phosphate could not be determined by the dye titration method. [c] The CMC was determined in 0.075 *M* NaCl with 0.01 *M* sodium phosphate buffer *p*H 7.0.

hyaluronic acid remaining) and time of reaction with enzyme for a mixture containing the standard 4 units of enzyme per cc. (curve 3) as well as for a mixture containing 8 units of enzyme per cc. (curve 1). When these experiments are repeated in the presence of 0.0003 *M* sodium dodecyl sulfate, such a linear relation between optical density and reaction time is no longer found (curves 4 and 2). It appears that the amount of enzyme activity decreases with time in the presence of detergent.

This conclusion is confirmed by kinetic experiments in which enzyme and detergent are incubated in the absence of substrate. The reaction between sodium dodecyl sulfate and hyaluronidase apparently approximates a first-order reaction with respect to enzyme at 38°, but follows a more complicated course at 30° (Fig. 3). Additional data were

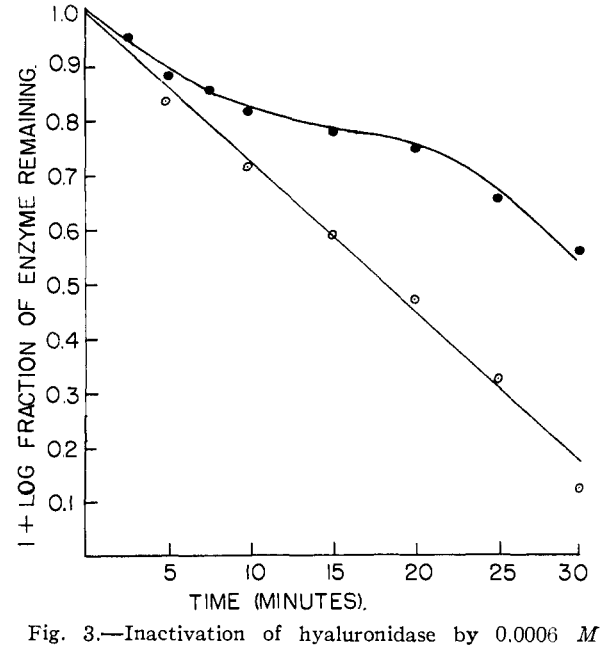


Fig. 3.—Inactivation of hyaluronidase by 0.0006 *M* sodium dodecyl sulfate: lower curve at 38°; upper curve at 30°.

obtained in similar kinetic experiments at 38° over a range of concentrations for each sulfated detergent. The concentrations which produced 50% inactivation of enzyme in 5 minutes are plotted in Fig. 4 *vs.* the CMC for each detergent. The least square equation (upper line) is $y = 0.95x$ with a standard error of estimate of 0.25. For purposes of comparison, the concentrations which produced 50% inactivation in the turbidity assay procedure were interpolated from Fig. 1 and were similarly plotted *vs.* CMC for each detergent. This least square equation (lower line) is $y = 0.83x - 0.09$ with a standard error of estimate of 0.17.

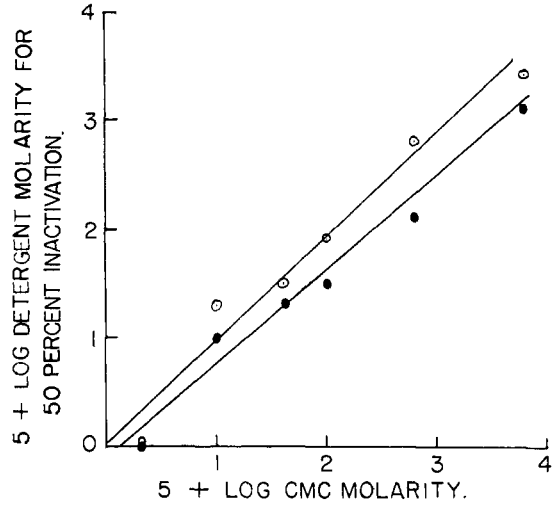


Fig. 4.—Correlation of effectiveness of detergents as inactivators and critical micelle concentration: ●, data from Fig. 1; O, data from kinetic experiments; values of CMC from Fig. 1.

Inactivation by Other Compounds.—In addition to detergents, various other compounds were investigated for their effect on hyaluronidase. The concentrations at which each compound causes a 50% inactivation of enzyme in the turbidity assay procedure are shown in Table I. When the compounds at the respective concentrations were incubated for 45 minutes with enzyme either under the ionic conditions of the turbidity assay or under the conditions of the kinetic experiments, only heparin and aurin tricarboxylic acid showed complete reversibility upon 100-fold dilution of the system. Sodium dodecyl sulfate and hexylresorcinol produced a time dependent loss of enzyme activity which was not reversible by 100-fold dilution or by addition of barium chloride to precipitate the detergent.

In other experiments, *d*-α-tocopheryl phosphate was incubated with 8 units of enzyme in 0.45% NaCl and 0.01 *M* phosphate *p*H 7.0 at 38° for various times up to 45 minutes, 1 cc. of hyaluronic acid

in 0.3 *M* phosphate *p*H 5.5 subsequently added, and the turbidity assay procedure carried on as described above. It was found that the extent of enzyme inhibition increased up to 100% with increase in the time of prior incubation of enzyme with *d*-α-tocopheryl phosphate. If, however, the above procedure was repeated with 800 units of enzyme in place of 8 units of enzyme, no detectable loss of added enzyme activity was observed upon 100-fold dilution of the system.

Discussion

Detergent Interactions.—It has been shown that the concentrations at which a series of straight chain sulfated alcohols are equally effective in inactivating hyaluronidase are correlated with the respective CMC's of the detergents. It is possible that the micelle is the kinetic unit which interacts with the enzyme.[19] Yet, inactivation by each detergent is observed to occur at concentrations well below the CMC. It would, therefore, appear necessary to assume the presence of some type of micelle in solutions with detergent concentrations lower than the CMC. However, only meager evidence has been adduced to date for the existence of such micelles ("McBain ionic micelles") in detergent solutions.[20]

An alternative and more plausible mechanism for enzyme–detergent interaction may be suggested. Present evidence indicates that, in general, the initial reaction of anionic detergent with a protein is stoichiometric with respect to the cationic groups of the protein. The combination is, however, more than electrostatic and is apparently stabilized by the mutual affinity of the hydrocarbon portions of adjacently bound detergent ions.[21] According to Lundgren,[22] excess detergent is attached by non-polar forces (presumed to be the same forces as those that bind detergent ions into micelles) to the detergent molecules stoichiometrically bound by electrostatic forces.

Anionic Polymers.—In the absence of precise knowledge of the molecular kinetics of the degradation of hyaluronic acid by hyaluronidase, it is difficult to devise unambiguous experiments to determine the nature of an observed inhibition. Nevertheless, it is not unreasonable to assume, as did McClean,[3] that the action of heparin upon hyaluronidase is a competitive inhibition. It differs from the action of detergents in that any combination of enzyme and inhibitor is not time-dependent and is fully reversible. Acetylated and nitrated hyaluronic acids have been found[6] to resemble heparin with respect to the absence of a time effect.

The action of aurin tricarboxylic acid is like that of heparin. It is significant, however, that aurin tricarboxylic acid is probably highly associated in solution as indicated by its failure to dialyze through a cellophane membrane readily permeable to small ions. This behavior is comparable to that shown by a large group of dyes known to be associated in solution.[23] Here it is reasonable to assume that the effective unit is a large colloidal aggregate. A similar assumption of molecular association would account for the inhibitory effectiveness of suramin and phosphorylated or sulfated hesperidin derivatives[24] and various compounds containing both hydroxyl and carboxyl groups.[7]

Hexylresorcinol.—The behavior of hexylresorcinol is similar to that of the detergents with respect to time-dependence and irreversibility of inactivation. It was demonstrated by the dye-titration method that hexylresorcinol forms micelles. Also, the effective concentration range of hexylresorcinol is closely related to its CMC. Thus, for the micelle forming substances (hexylresorcinol, the straight-chain sulfated alcohols, and sodium di-(2-ethylhexyl)-sulfosuccinate) details of chemical structure appear less significant for enzyme inactivation than do the more general colloidal properties which determine molecular association.

Tocopheryl Phosphate.—*d*-α-Tocopheryl phosphate shows a measurable time rate of inactivation of hyaluronidase. In this respect, it resembles the micelle-forming substances as might have been predicted on chemical structural grounds. A CMC could not be determined by the dye-titration method, possibly because of the extremely low solubility of the compound. Also, the inactivation of the enzyme was completely reversed upon dilution. It is possible, however, that the mechanism of inactivation is similar to that of the sulfated detergents. Similar results were obtained by Miller and Dessert,[9] who failed, however, to recognize that the inactivation of enzyme by inhibitor was a continuing action with time.

Other Enzymes.—Sulfated hydrocarbon carcinogens inhibit[25] various enzymes in animal tissue homogenates. A large number of enzymes, notably urease, invertase, peroxidase and malt amylases are inhibited[26] by sodium dodecyl sulfate (at minimal concentrations which are estimated to be near the CMC) provided the *p*H is sufficiently low. Thus, 0.001 *M* sodium dodecyl sulfate completely inhibits urease (isoelectric at *p*H 5.0 to 5.1) at *p*H 5.0 but has no effect at *p*H 5.4. It was also reported[27] that β-amylase is inactivated by sodium dodecyl sulfate below *p*H 5.1 but not at all above this *p*H. The effective concentration range found agrees remarkably well with that given for hyaluronidase in Fig. 1. Further, the degree of inactivation of β-amylase was dependent upon time of incubation and was apparently irreversible. Heparin was also an inhibitor of the enzyme.

Hyaluronidase differs from the above enzymes principally in that detergent inactivation proceeds well above the isoelectric point[28] of 5.7. This high affinity for anions when the net charge on the pro-

(19) The idea that the micelle interacts as an intact unit with protein has been advanced (D. G. Dervichian, *Disc. Faraday Soc.*, **6**, 7 (1949)) with some supporting evidence from calorimetric data (B. A. Pethica, *ibid.*, **6**, 237 (1949)) and other observations (E. G. Cockbain, *Trans. Faraday Soc.*, **49**, 104 (1953)) on protein–detergent interactions in the region of large detergent–protein ratios.

(20) J. W. McBain, "Colloid Science," D. C. Heath and Co., Boston, Mass., 1950. Chapter 17.

(21) F. W. Putnam, *Adv. Protein Chem.*, **4**, 79 (1948).

(22) H. P. Lundgren, *Textile Research J.*, **15**, 335 (1945).

(23) A. E. Alexander and P. Johnson, "Colloid Science," Oxford University Press, Cambridge, 1949.

(24) J. M. Beiler and E. Martin, *J. Biol. Chem.*, **171**, 507 (1947); **174**, 31 (1948).

(25) D. Hockenhull, *Nature*, **162**, 813, 850 (1948).

(26) E. D. Wills, *Biochem. J.*, **53**, XX (1953).

(27) K. Myrbäck and B. Persson, *Ark. Kemi*, **4**, 531 (1952).

(28) L. Hahn, *Biochem. Z.*, **315**, 83 (1943).

tein is negative may well be related to enzyme structural requirements for combination with the highly charged natural substrate molecules. In spite of this difference, future detailed investigations of other enzyme systems may reveal a relationship between micellar properties of detergents and their inactivating action similar to that found for hyaluronidase.[29]

(29) The importance of micellar properties of cationic detergents is indicated by observations that the ability of a cationic detergent to accelerate the enzymatic decarboxylation of glutamate and glutamine parallels its ability to form micelles (D. E. Hughes, *Biochem. J.*, **45**, 325 (1949); *ibid.*, **46**, 231 (1950)). It was supposed that the cationic agent removes an inhibitor and thus increases the rate of action of enzyme. The concentration for maximum acceleration approximately coincided with the CMC for each member of a series of detergents.

Acknowledgment.—The author would like to express his appreciation to Dr. Albert Dorfman for many helpful discussions.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

Reactions of Hindered α-Substituted Succinic Acids. III. Mechanism of the Reaction of the Haloacids with Bases[1]

BY WYMAN R. VAUGHAN AND RANDEL Q. LITTLE, JR.[2]

RECEIVED NOVEMBER 9, 1953

The mechanism of the elimination of the β-carboxyl together with halide ion of hindered α-halosuccinic acids as a consequence of reaction with bases is discussed. The fact that this reaction may occur by a unimolecular process with either the *cis* or *trans* configuration of the two groups is considered, and the nature of the competing reaction in each instance is examined.

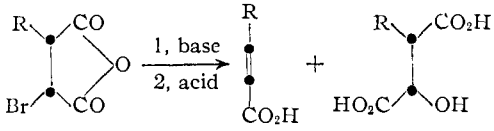
In the first paper in this series[3] the general characteristics of the reactions of 2-bromo-3-methyldibenzo [2,2,2]bicycloöctadiene-2,3-*cis*-dicarboxylic anhydride (I), 2-bromodibenzo[2,2,2]bicycloöctadiene-2,3-*cis*-dicarboxylic anhydride (II) and 2-bromodibenzo[2,2,2]bicycloöctadiene-2,3-*trans*-dicarboxylic acid (III) with alkali were described. The elimination of the β-carboxyl with bromide ion was observed with all three compounds, and with I and II the usual formation of the α-hydroxy-

I, R = CH_3
II, R = H

III, X = Br
V, X = OH

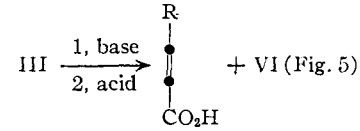
Fig. 1.

acid by β-lactone formation with cleavage on the carbonyl to give the *trans*-configuration was noted. With III the accompanying reaction was an unusual Wagner–Meerwein rearrangement, the nature



I and II

of which has been considered in the preceding paper of this series.[4]



The present investigation was designed to shed more light on the general characteristics of the behavior of β-haloacids in alkaline media.

It has been established that in neutral solutions the hydrolysis of both α-haloacids[5] and β-haloacids[6] proceeds with retention of configuration, whereas in acidic or alkaline media the resultant hydroxy acids have the inverted configuration. With the α-haloacids an intermediate α-lactone or lactonic transition state has been proposed, and support for this hypothesis is derived from the observation that the lithium alumino-hydride reduction of optically active 2-chloro-2-phenylpropionic acid affords as a by-product optically active 2-phenyl-1,2-propanediol with complete configurational inversion.[7] The non-inverting hydrolysis follows a first-order path and is *p*H independent while the alkaline, inverting hydrolysis is first order in the hydroxyl ion.[5]

With the β-haloacids the isolation of the β-lactone has actually been achieved,[8] and the chemistry of the β-lactone system clearly indicates the nature of the inverting and non-inverting reactions.[6] Thus both the α-haloacid and β-haloacid hydrolyses may be described in similar terms.

(1) Abstracted from a portion of the Ph.D. dissertation of Randel Q. Little, Jr., University of Michigan, 1953. For the preceding paper in this series see THIS JOURNAL, **76**, 1748 (1954).

(2) American Brake Shoe Company Fellow, 1952–1953.

(3) W. R. Vaughan and K. M. Milton, THIS JOURNAL, **74**, 5623 (1952).

(4) W. R. Vaughan, M. V. Andersen, Jr., and R. Q. Little, Jr., *ibid.*, **76**, 1748 (1954).

(5) W. A. Cawdrey, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 1208 (1937); *cf.* L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 175–178.

(6) A. R. Olson and R. J. Miller, THIS JOURNAL, **60**, 2687 (1938); A. R. Olson and J. L. Hyde, *ibid.*, **63**, 2459 (1941); *cf.* P. D. Bartlett and R. N. Rylander, *ibid.*, **73**, 4275 (1951).

(7) E. L. Eliel and J. P. Freeman, *ibid.*, **74**, 923 (1952).

(8) H. Johannson, *Ber.*, **48**, 1262 (1915).