by centrifugation of homogenates at 120,000 × *g.* for 30 minutes, when added to washed mitochondria, restored the activity to essentially the level originally obtained in the crude system.

Both the crude and partially resolved systems require the addition of diphosphopyridine nucleotide (DPN) and nicotinamide. Triphosphopyridine nucleotide (TPN) does not replace the DPN requirement. The over-all oxidation process shows a partial dependence on the presence of adenylic acid (AMP) although this phenomenon may be related to the stabilizing effect of adenylic acid and adenosinediphosphate on the general metabolic integrity of mitochondria.[5]

As shown in Table I, dialysis of the soluble fraction of the system does not lead to a significant loss in activity, nor can this fraction be replaced by a concentrated, boiled extract of whole liver or liver fractions. The inactivity of either the mitochondrial or the soluble fractions alone, and the demonstration of a considerable lag phase in the appearance of $C^{14}O_2$ suggests, as one possibility, the accumulation of an intermediary compound derived from the side chain carbon atoms which is subsequently oxidized by the terminal oxidizing systems of the mitochondrial elements.

LABORATORY OF CELLULAR PHYSIOLOGY, NATIONAL HEART INSTITUTE
NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY CHRISTIAN B. ANFINSEN
BETHESDA 14, MARYLAND MARJORIE G. HORNING

RECEIVED JANUARY 23, 1953

ENZYMATIC CLEAVAGE OF THE CITROVORUM FACTOR

Sir:

In our studies with soluble enzyme preparations which liberate bound forms of folic acid from liver, we observed that citrovorum factor (CF) added to such preparations disappeared at a rapid rate. We now have obtained from horse liver a protein fraction which, in the presence of *l*-glutamic acid, effectively destroys CF.

The protein fraction was obtained as the 30% saturated (0°) ammonium sulfate precipitate from a cold water extract (0°) of horse liver.

The influence of *l*-glutamic acid on the rate of inactivation of CF by the liver fraction is shown by the data in Table I. The loss of CF activity as measured by both *Leuconostoc citrovorum* and *Streptococcus faecalis* R, is paralleled by a rise in arylamines indicating a cleavage of the pteridine moiety from the *p*-aminobenzoylglutamic acid residue.

TABLE I

RECOVERY OF CF AFTER INCUBATION WITH LIVER PROTEIN FRACTION

Incubated at 37° for 2 hr. in 0.08 *M* Na_2HPO_4; volume, 7 ml.; initial CF = 56 γ.

L-Glutamic acid, *M*	None	0.001	0.002	0.004	0.0075	0.01
CF, γ	51.0	36.8	31.2	26.6	24.2	23.0

The role of *l*-glutamic acid appears to be specific. Other amino acids including *l*-glutamine and also known metabolic products of *l*-glutamic acid do not replace *l*-glutamic acid in this system. It is significant that *p*-aminobenzoic acid inhibits the reaction (31% inhibition with 0.01 *M* concentration of *l*-glutamic and *p*-aminobenzoic acids). While CF is attacked readily by the protein fraction in the presence of *l*-glutamic acid, neither pteroylglutamic acid nor its N-10-formyl derivative is affected.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL JOHN C. KERESZTESY INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY MILTON SILVERMAN BETHESDA, MARYLAND

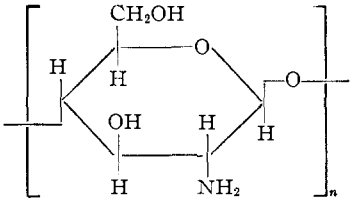
RECEIVED FEBRUARY 20, 1953

DIRECT EVIDENCE OF THE INFLUENCE OF SULFAMIC ACID LINKAGES ON THE ACTIVITY OF HEPARIN-LIKE ANTICOAGULANTS

Sir:

Heparin, the naturally occurring glucosamine-glucuronic acid polysaccharide polysulfate, is characterized by its high anticoagulant activity (U.S.P. Heparin is defined as having not less than 100 International Units per mg.) and by its essentially nontoxic nature (mouse intravenous LD_{50} = 1500–2000 mg./kg.[1]). Extensive work by numerous investigators has indicated that the activity of heparin is dependent, among other things, upon the degree of sulfation of the molecule and recently both Jorpes[2] and Meyer[3] concluded, as earlier considered a possibility[4] and more recently affirmed[5] by Wolfrom, that the amino groups in the molecule are sulfated and demonstrated that hydrolysis of the protected amino linkages resulted in essentially complete inactivation of the material. In order to test the validity of the postulate that the presence of sulfamic acid groups is a major factor essential for the high activity and presumably for the low toxicity of heparin, and also because the stated conclusions were based largely upon indirect evidence, we undertook to obtain direct evidence of the contribution of sulfamic acid linkages to the anticoagulant activity of polysaccharide polysulfate esters of the heparin type.

In this work the polyglucosamine, chitosan



was used as a model substance in sulfation experiments designed for the preparation of products in which the amino and hydroxyl groups were sulfated to varying degrees. Some of the data obtained on some of these products are given in Table I.

Thus, for the first time, there are data which indicate a correlation in agreement with the hypothesis

(1) J. Seifter and A. J. Begany, *Am. J. Med. Sci.*, **216**, 234 (1948).
(2) J. E. Jorpes, H. Boström and V. Mutt, *J. Biol. Chem.*, **183**, 607 (1950).
(3) K. H. Meyer and D. E. Schwartz, *Helv. Chim. Acta*, **33**, 1651 (1950).
(4) M. L. Wolfrom and W. H. McNeely, THIS JOURNAL, **67**, 748 (1945).
(5) M. L. Wolfrom, R. Montgomery, J. V. Karabinos and P. Rathgeb, *ibid.*, **72**, 5796 (1950).

TABLE I

Sample	A	B	C	D	E
η_{sp}/c, c = 1.00% in 0.50 N NaCl, 30°	0.328	0.358	0.370	0.200	0.380
S, %	16.17	16.74	14.28	14.27	15.99
N (Kjeldahl), %	3.75	3.44	3.70	3.12	3.15
N (Van Slyke), %	1.02	0.25	1.09	0.19	0.17
Moles of $-SO_3Na$ group per repeating unit					
Total	1.68	1.80	1.32	1.32	1.64
on OH	0.95	0.87	0.61	0.38	0.69
on NH_2	0.73	0.93	0.71	0.94	0.95
Activity, I.U./mg.	13	59	11	57	57

that, other conditions being equal, the contribution of sulfamic acid groups (above a certain limit) to the anticoagulant activity of polysaccharide polysulfate esters is far greater than that of sulfate ester groups.

That there is no simple relationship, *per se*, between anticoagulant activity and acute toxicity is amply demonstrated by other of our sulfated chitosan products, shown in Table II.

TABLE II

Sample	F	G	H	I
Activity, I.U./mg.	20	57	63	74
LD_{50}, i.v. in mice, mg. of kg.	3250 ±250	1500 ±500	1700 ±300	1250 ±250

In consideration of reports that the U.S.P. method does not give a reliable measure of *in vivo* activity with polysaccharide polysulfate synthetic anticoagulants,[6,7,8] our products which have been assayed by the U.S.P. method are currently being evaluated for activity by *in vivo* methods.

(6) E. G. Snyder (to Wyeth Inc.), U. S. Patent 2,508,433, May 23, 1950.

(7) C. N. Mangieri, R. Engelberg and L. O. Randall, *J. Pharmacol. Exptl. Therap.*, **102**, 156 (1951).

(8) H. E. Stavely, P. J. Baker, Jr., and H. G. Payne, *Federation Proc.*, **11**, 488 (1952).

WARNER-CHILCOTT RESEARCH LABORATORIES JOHN DOCZI
113 WEST 18TH STREET ALEX FISCHMAN
NEW YORK 11, NEW YORK JOHN A. KING

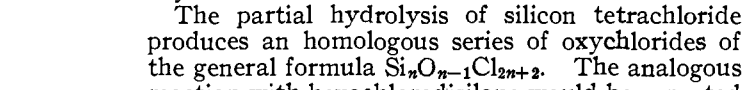
RECEIVED JANUARY 29, 1953

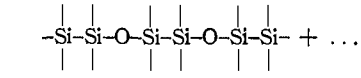
THE PARTIAL HYDROLYSIS OF HEXACHLORODISILANE

Sir:

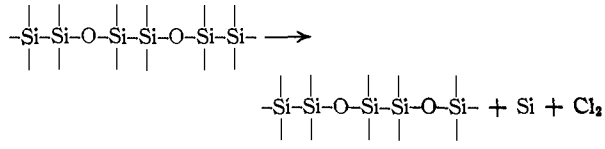
Part of the current research program of these laboratories involves a comparative study of the partial hydrolysis, ammonolysis and thiohydrolysis of silicon halides. Using the method of Schumb and Stevens,[1] we have succeeded in partially hydrolyzing hexachlorodisilane, obtaining the first member of what is believed may be a new series of silicon oxychlorides.

The partial hydrolysis of silicon tetrachloride produces an homologous series of oxychlorides of the general formula $Si_nO_{n-1}Cl_{2n+2}$. The analogous reaction with hexachlorodisilane would be expected similarly to give a series of the type $Si_{2n+2}O_nCl_{4n+6}$, as indicated by the schematic arrangement





The first member of this series, Si_4OCl_{10}, has been isolated and identified. Evidence also has been obtained for the existence of higher members, which, however, appear to undergo thermal decomposition during fractional distillation. This decomposition is probably the result of the thermal instability of long chains containing Si–Si linkages and has prevented the isolation of higher members. Analyses and estimated molecular weights of higher boiling fractions, however, are in the expected region. One of these fractions appears to be a decomposition product of the second member of the series referred to above, by such a process as the following



The hexachlorodisilane was added to dry ether in a three-necked round-bottom flask fitted with a slip-seal stirrer. The solution was cooled to −78° in a solid carbon dioxide–trichloroethylene bath and, with constant stirring, a measured amount of water was added dropwise from a buret. The mixture remained in the cold-bath for two hours and was then allowed to come to room temperature. The ether and unreacted hexachlorodisilane were removed by fractional distillation. The partial hydrolysis was then repeated with the recovered hexachlorodisilane. In a series of six such reactions, a total of 230 g. of Si_2Cl_6 was partially hydrolyzed.

The higher boiling residues remaining after the removal of the ether and unreacted hexachlorodisilane were combined and fractionated under reduced pressure. During the fractionation the contents of the distillation pot darkened gradually and a considerable quantity of black residue remained after removal of all liquid material. All fractions were clear liquids, increasing in viscosity with increasing temperature. While the first two fractions exhibited relatively narrow boiling point ranges, no well defined holds were observed at higher temperatures.

The first fraction, b.p. 120–123° (13 mm.), weighed about 12 g. *Anal.* Calcd. for Si_4OCl_{10}: Si, 23.3; Cl, 73.4; Si–Si bonds, 2/mole; mol. wt., 483. Found: Si, 23.2; Cl, 73.4; Si–Si bonds, 1.96/mole; mol. wt., 475. The second fraction, b.p. 140–143° (14 mm.), weighed about 5 g. *Anal.* Calcd. for $Si_5O_2Cl_{12}$: Si, 23.5; Cl, 71.2; Si–Si bonds, 2/mole; mol. wt., 598. Found: Si, 23.4, Cl, 71.0, Si–Si bonds, 1.93/mole; mol. wt., 595. The number of Si–Si linkages was determined by measuring the volume of hydrogen resulting from decomposition of the sample with dilute alkali while molecular weights were obtained from the freezing point depression of *p*-dioxane.

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INST. OF TECH. W. C. SCHUMB
CAMBRIDGE, MASSACHUSETTS R. A. LEFEVER

RECEIVED MARCH 4, 1953

(1) W. C. Schumb and A. J. Stevens, THIS JOURNAL, **72**, 3178 (1950).