amount of ATP and is independent of CoA, thus suggesting that succinyl phosphate may be formed, (d) in experiments with sulfanilamide,[10] a CoA- and ATP-dependent decrease in free amide is observed; this decrease is greater if carbon dioxide production is inhibited, suggesting that succinyl-CoA, as well as succinyl phosphate, may be formed. A detailed treatment of the experimental results and of the postulated mechanism of succinate activation will be presented elsewhere.

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(11) Part of this work was done at the Hopkins Marine Station, Pacific Grove, California, on an Atomic Energy Commission Postdoctoral Fellowship.

SULFATED NITROGENOUS POLYSACCHARIDES AND THEIR ANTICOAGULANT ACTIVITY[1]

Sir:

N-Deacetylated chitin, previously swollen with pyridine, was heterogeneously sulfated at 100° for 1 hour with chlorosulfonic acid and pyridine to yield a product, isolated (inorganic salts were removed by dialysis) as the amorphous, water-soluble sodium salt, containing essentially two N-sulfate and one O-sulfate groups per anhydrodisaccharide unit; $[\alpha]^{25}D$ −23° (*c* 1.5, water). *Anal.* Calcd. for $C_{12}H_{19}O_7(NSO_3Na)_2(OSO_3Na)$: C, 22.93; H, 3.05; N, 4.46; S, 15.31; Na, 10.98. Found: C, 22.68; H, 3.08; N, 4.02; S, 15.6; Na, 11.2; $-NH_2$ (by ninhydrin), absent; NAc, absent. This preparation exhibited the behavior in the Van Slyke amino acid assay characteristic of the acid-labile N-sulfate group present in heparin.[2,3] Its anticoagulant activity was 56 International Units (I. U.)/mg. The animal (mouse intravenous) toxicity was approximately double that of heparin, a finding believed to be due to the unsuitably high molecular size of the substance.

(1) Supported by the Bristol Laboratories, Inc., Syracuse, N. Y., (R. F. Project 432).

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Chondroitinsulfuric acid (from cartilage) was essentially homogeneously N-deacetylated with 45% NaOH (25, 48 hr.) under nitrogen and in the presence of antioxidants (benzyl alcohol and sodium sulfite) and was sulfated as described above (but at 80–90°). The product was isolated as the amorphous sodium salt and is under further analytical characterization; $-NH_2$ (by ninhydrin), absent. The anticoagulant activity was 48 I. U./mg. The same sample of sodium chondroitin sulfate was subjected to the above sulfation procedure without preliminary N-deacetylation and the product, isolated in the same manner, showed an anticoagulant activity of *ca.* 10 I. U./mg. Sodium heparinate was re-sulfated under these conditions with a reduction in its activity from 110 to 55 I. U./mg. and an increase in the sulfur content from 12 (initial) to 14.4%.

Methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride[4] was sulfated as above to produce the amorphous, water-soluble barium salt of the N-sulfate, tri-O-sulfate; $[\alpha]^{25}D$ +4° (*c* 3.4, H_2O). *Anal.* Calcd. for $C_7H_{11}NO_{17}S_4Ba_2 \cdot 2H_2O$: S, 15.63; Ba, 33.48. Found: S, 15.64; Ba, 32.98. A 3×10^{-4} *M* solution of this substance in 0.004 *N* HCl at 95° lost 1.0 mole of sulfate in ≦20 min. with the concomitant release of the free amino group (ninhydrin). The O-sulfate was removed relatively more slowly and only completely so after 12 hr. Previously reported results[2] on the inactivation of heparin by mild acidity were considered to involve a negligible sulfate loss. On the basis of our present knowledge of the heparin molecule,[5] this sulfate loss is about equivalent to the amino group released so that a sulfate group shift[5] is not a required postulation.

The above results show that the sulfamic acid group is a potent contributor to anticoagulant activity. Experiments are now underway to determine the optimum molecular size for these chemically modified polysaccharides.

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