

Short Communications

On the Linkage of the Amino Group in Heparin

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In a previous paper¹ it was shown that one of the sulfate groups in heparin must be linked to the amino group in a $-NH_2SO_3H$ linkage giving rise to a substituted amidosulfuric or sulfamic acid. Since this linkage as shown by Berglund² who first described the properties of the amidosulfuric acids, is quite stable in alkaline solution whereas the acetyl component of the aminoacetyl group, $-NH-Ac$, is easily split off, heparin and chondroitin sulfuric acid should behave differently when submitted to treatment with alkali. This could also be shown to be the case.

Less than 10 per cent of the total nitrogen of heparin is liberated as ammonia during treatment with N NaOH for two hours at $100^\circ C$ while about 55 per cent of the total nitrogen of the chondroitin sulfuric acid are liberated under similar conditions.

Experimental. 200 mg lots of heparin and of chondroitin sulfuric acid were dissolved in 100 ml N NaOH in a 250 ml three-necked round flask with a Liebig's cooler, a thermometer and a glass capillar and heated to boiling on a metal bath. Nitrogen was continuously bubbled through the flask with a speed of about 2 l per minute and the ammonia formed was taken up in Erlenmeyer flasks, supplied with 5 ml of a 2 per cent boric acid solution. The

Table 1. Analysis of the material used.

Preparation	Per cent of dry substance			
	N	S	Ash	Amino-N
I. Glucosamine HCl	6.31	—	0.49	—
II. Sodium salt of chondroitin sulfuric acid	2.19	6.69	22.94	0.02
III. Sodium salt of heparin	2.99	12.76	43.87	0.20
IV. Sodium salt of heparin partly hydrolyzed with 0.04 N HCl	2.93	10.29	18.20	1.27

flasks were changed at different intervals. The amount of ammonia distilling over was estimated by titration with 0.01 N HCl using the ordinary Kjeldahl indicator.

The analyses of the preparations used are given in Table 1.

Preparation IV of the table was obtained in the following way.

1 g of sodium salt of heparin (preparation III) was dissolved in 40 ml 0.04 N HCl and hydrolyzed on a boiling water bath for 8 hours. After cooling the hydrolysate was dialyzed against running tap water overnight, concentrated *in vacuo* to a thick syrup, precipitated with 10 volumes of alcohol and treated with absolute alcohol and ether. Yield 600 mg.

The results are shown in Fig. 1. In the diagram the amount of ammonianitrogen liberated expressed in per cent of total nitrogen is plotted against time of hydrolysis in minutes.

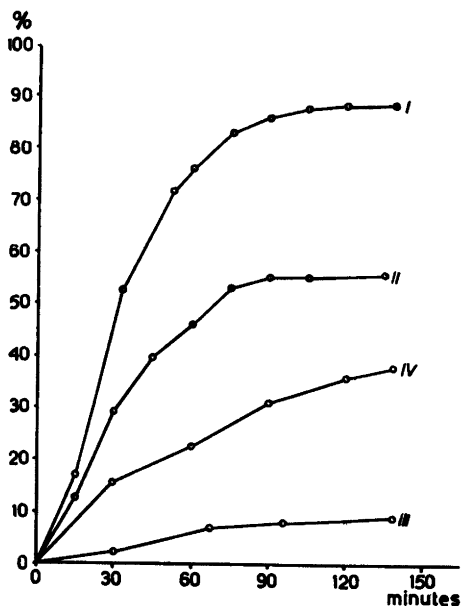


Fig. 1. Liberation of ammonia when heparin, chondroitin sulfuric acid and glucosamine are treated with N NaOH at $100^{\circ}C$. Distilled nitrogen in per cent of total nitrogen is plotted against time in minutes.

Curve I. Glucosamine hydrochloride.

Curve II. Sodium salt of chondroitin sulfuric acid.

Curve III. Sodium salt of heparin.

Curve IV. The same salt of heparin with about 60 per cent of the amino group free after 8 hours hydrolysis at $100^{\circ}C$ with $0.04 N$ HCl.

In glucosamine (curve I) where the amino group is free, the formation of ammonia took place rapidly. After 135 minutes 90 per cent of the total nitrogen had distilled over as ammonia.

In chondroitin sulfuric acid (curve II) where the amino group is acetylated, the formation of ammonia was also remarkably rapid. After 135 minutes 55 per cent of the total nitrogen had distilled over.

In heparin (curve III) the formation of ammonia was very poor. After 135 minutes only about 10 per cent of the total nitrogen had distilled over as ammonia.

If the amino group in heparin, however, had been partly liberated by means of slightly acid hydrolysis with $0.04 N$ HCl the formation of ammonia was quite considerable (curve IV). After 135 minutes 38 per cent of the total nitrogen had distilled over as ammonia.

Consequently the linkage of the amino group in heparin is of another type than in the chondroitin sulfuric acid. The high resistance to alkaline hydrolysis may be considered as a further support for the assumption that the amino group in heparin is linked to sulfuric acid.

This investigation was aided by grants from the Wallenberg Foundation and the Vitrum Company, Stockholm.

1. Jorpes, E., Boström, H., and Mutt, V. *J. Biol. Chem.* **183** (1950) 607.
2. Berglund, E. *J. Chem. Soc.* **34** (1878) 643.

Received January 29, 1952.

Surface Balance Studies of the Multimolecular Layers of Isodextropimaric Acid

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The properties of isodextropimaric acid monolayers on water have been recently studied with a surface balance^{1,*}. It was found that the surface pressure in the isodextropimaric acid monolayer began to increase on compression when the area per molecule was reduced to 54.1 \AA^2 , and the monolayer collapsed when the area was 42.6 \AA^2 per molecule. After this an equilibrium surface pressure equal to 19.7 dynes per cm was established, the layer remained liquid.

* A paper read at the VII Nordiska kemistmötet in Helsingfors, August, 1950.