# A NEW TYPE OF HEPARIN "ω-HEPARIN" ISOLATED FROM WHALE ORGANS

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The acid mucopolysaccharide isolated from lung and intestine of finback whale (Balaenoptera physalus) by a modification of the procedure of Kuizenga and Spaulding (1943) is proved in this communication to be a new type of heparin. It is therefore designated tentatively as "ω-heparin." In a previous paper (Hashimoto et al., 1963), this substance was shown to be more than twice as active in the anticoagulant assay as α-heparin prepared by the same procedure from the bovine lung. Lipolytic activity of the former was, however, found to be similar or somewhat less than that of the latter.

The present note describes preliminary results of a study on the elucidation of the chemical nature of ω-heparin.

ω-Heparin (Preparation Lot No. 6205), kindly supplied by Dr. T. Shibata, appeared essentially homogeneous on electrophoresis and ultracentrifugation in pH 8.0 phosphate and pH 4.6 acetate buffers. The aminosugar in this preparation was found to be glucosamine, and uronic acid was identical with that of α-heparin as judged by paper chromatography. The quantitative analytical data (Table I) revealed that ω-heparin contained much less sulfur (sulfate) than α-heparin and the former had higher dextrorotation than the latter. A part of the glucosamine of ω-heparin obtained by the mild acid hydrolysis could not be determined as 2,5-anhydromannose by deamination with nitrous

acid. In contrast, the amount of glucosamine of α-heparin produced under the same condition was much more than that liberated by strong acid hydrolysis. These observations suggested that the portion of glucosamine of ω-heparin that could not be deaminated was not N-sulfated. Moreover, infrared spectral analysis of ω-heparin showed an absorption at 1560 $cm^{-1}$ in addition

TABLE I

ANALYTICAL DATA OF ω-HEPARIN (WHALE) AND α-HEPARIN (BOVINE)

| Analysis | ω-Heparin | α-Heparin |
|---|---|---|
| Nitrogen (%) | 2.5 | 2.2 |
| Glucosamine[a] (%) | 30.4 | 26.3 |
| N-Desulfated glucosamine[b] (%) | 26.0 | 31.6 |
| Glucuronic acid[c] (%) | 50.2 | 44.2 |
| Sulfur[d] (%) | 9.0 | 13.3 |
| Ash (as Na) (%) | 13.1 | 15.1 |
| $[\alpha]_D^{20}$ (degree) | +65.4 | +48.7 |

a) The amount of glucosamine in the hydrolyzates with 4N HCl at 100°C for 4 hours (Yosizawa, 1961).

b) The amounts of glucosamine which could be determined as 2,5-anhydromannose by the direct deamination with nitrous acid after being treated with 20% acetic acid at approximately 28°C for 72 hours.

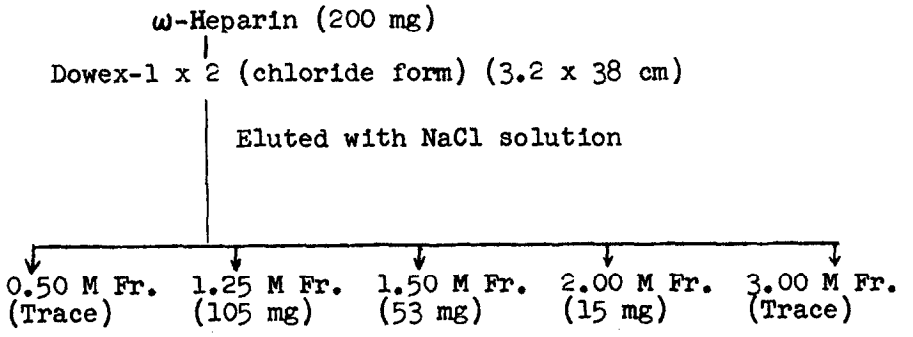
c) Determined by the carbazole method of Dische (1947).

d) Analyzed by the procedure of Antonopoulos (1962).

to the absorptions of α-heparin suggesting that the preparation of ω-heparin contained N-acetylglucosamine. To determine whether ω-heparin was contaminated with other mucopolysaccharides comprising N-acetylglucosamine, the preparation was fractionated on a column of Dowex-1 by the procedure of Schiller et al.(1961).

TABLE II

FRACTIONATION OF ω-HEPARIN AND ANALYTICAL DATA ON THE FRACTIONS




| Analysis | 1.25 M Fr. | 1.50 M Fr. | 2.00 M Fr. |
|---|---|---|---|
| Nitrogen (%) | 2.6 | 2.6 | 2.3 |
| Glucosamine[a] (%) | 30.5 | 30.2 | 27.0 |
| N-Desulfated glucosamine[b] (%) | 26.8 | 25.3 | 18.6 |
| Glucuronic acid[c] (%) | 50.5 | 49.2 | 37.1 |
| Sulfur[d] (%) | 8.9 | 9.1 | 8.5 |
| Ash (as Na) (%) | 13.0 | 13.7 | 10.8 |

a), b), c), d) See Table I

The results (Table II) revealed that ω-heparin was eluted mainly in 1.25 M and 1.50 M fractions. Moreover, these fractions showed identical infrared absorption spectra with that of the original material. These findings indicated, therefore, that N-acetylglucosamine must be an integral component of ω-heparin. Approximately one-fourth of glucosamine in ω-heparin was calculated to be N-acetylated and the remaining was N-sulfated (Table III).

To determine the position of N-acetylglucosamine in the molecule, the N-desulfated ω-heparin was deaminated with nitrous acid and then dialyzed. The non-dialyzable fraction, designated

TABLE III

CONTENT OF ACETYL AND GLUCOSAMINE IN THE FRACTIONATED ω-HEPARIN (Expressed in Percent)

| Component | Original Material | 1.25 M Fr. | 1.50 M Fr. | 2.00 M Fr. |
|---|---|---|---|---|
| Acetyl* | 1.96 | 1.93 | 2.01 | 2.92 |
| Glucosamine | | | | |
| (N-Desulfated glucosamine[b]) | 26.0 | 26.8 | 25.3 | 18.6 |
| (Equivalent amount of glucosamine to acetyl content (calcd.) | 8.2 | 8.0 | 8.4 | 12.2 |
| (Total amount (calcd.) | 34.2 | 34.8 | 33.7 | 30.8 |

*Estimated by the procedure of Ludowieg and Dorfman (1960).
b)See Table I.

as deamination product, was precipitated with ethanol, yielding approximately 18% of the starting material. The analytical data of this product (Table IV) showed that all glucosamine residues were N-acetylated. Furthermore, equivalent amounts of 2,5-anhydromannose and glucosamine were detected in the deamination product. These findings indicate that the deamination product was constituted of an N-acetylglucosamine-containing polysaccharide core carrying fragments which possessed the equimolar 2,5-anhydromannose to N-acetylglucosamine.

The average molecular weight of ω-heparin was calculated to be approximately 10,000 using a value of 0.50 (Creeth and Record, 1952) for the partial specific volume of heparin. This molecular weight is similar to that of α-heparin (approximately 9,000) prepared by the same method, but markedly different from that of heparitin sulfate (Brown, 1957). These observations sug-

TABLE IV

ANALYTICAL DATA ON THE DEAMINATION PRODUCT OF ω-HEPARIN

| Analysis | % | Ratio |
|---|---|---|
| Glucosamine[a)] | 18.2 | 1.00 |
| Glucuronic acid[c)] | 30.1 | 1.53 |
| Sulfur[d)] | 5.1 | 1.56 |
| Acetyl* | 4.4 | 1.01 |
| 2,5-Anhydromannose** | 18.1 | 1.10 |
| Ash (as Na) | 11.3 | - |

a), c), d) See Table I

* See Table III

** Determined directly with indole-HCl reagent.

gest that ω-heparin may be visualized as being built of a core which consists of an N-acetylglucosamine-containing polysaccharide and to which α-heparin-type polysaccharides are attached. A specific three-dimensional shape is thus proposed for the structure of ω-heparin. Further, it could be speculated that the high anticoagulant activity of ω-heparin might be due to the specific shape of the molecule.

Although certain preparations of heparin or heparin-like substances such as mactins A and B contained acetyl residues, it was indicated that the nitrogen atoms in these preparations were not acetylated (Burson et al., 1956). The acetyl residues in these substances were shown to be due to a contaminant(s) (cf. Wolfrom, 1958). In case of ω-heparin, however, the acetyl residues were confirmed to be the integral component of the molecule.

The anticoagulant activity of both ω-heparin and α-heparin was destroyed by N-desulfation, but the lipolytic activity was

not affected. The deamination product of ω-heparin did not show these biological activities. These findings indicate that the moieties responsible for the anticoagulant activity of heparin were different from those for the lipolytic activity. Thus, it was suggested that N-sulfates in heparin are responsible for the former and certain other acidic groupings for the latter activity.

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