Chemistry and Biology of Heparin

Editors:
Roger L. Lundblad
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Harold R. Roberts

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CHEMISTRY AND BIOLOGY OF HEPARIN

Proceedings of the International Conference on the Chemistry and Biology of Heparin held in Chapel Hill, North Carolina, U.S.A. on March 20-22, 1980

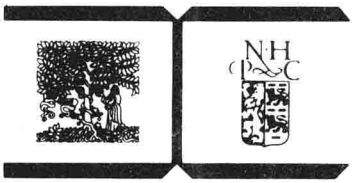
Editors:

ROGER L. LUNDBLAD, Ph.D.
Professor of Pathology and Biochemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.

W. VIRGIL BROWN, M.D.
Professor of Medicine, Mount Sinai School of Medicine, New York, New York, U.S.A.

KENNETH G. MANN, Ph.D.
Hematology Consultant, Mayo Clinic and Foundation, Rochester, Minnesota, U.S.A.

HAROLD R. ROBERTS, M.D.
Professor of Medicine and Pathology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.



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PREFACE

A conference on The Chemistry and Biology of Heparin was held in Chapel Hill, North Carolina on March 20-22, 1980. The impetus for this meeting evolved from a number of informal discussions between the organizers of this conference. In the course of the planning for this meeting, several investigators asked - why another meeting on heparin? - there have been at least five such meetings within the past decade. Our answer to this question was two-fold. First, the area of heparin research has move forward with great speed during the past five years such that it was appropriate to convene a number of distinguished investigators with varied approaches to this problem for an assessment of the "state of the art". Secondly, it was our opinion that a comprehensive meeting was necessary to do justice to the many facets of this interesting molecule.

With above considerations in mind, we then sought to identify specific areas of major concern regarding heparin. Once having identified major "target" areas, we invited investigators who had contributed to the heparin literature during the past five years to participate in this conference. The response to this invitation was overwhelming and resulted in an exhausting schedule to two and half days. We would take this opportunity to thank the participants in this conference for their patience and complement them on their endurance.

Finally, the conference would have not been a success without the support of the Councils on Thrombosis and Arteriosclerosis of the American Heart Association and the North Carolina Affiliate of the American Heart Association. It is with particular pleasure that we acknowledge the support of Drs. A. Bleakley Chandler of the Medical College of Georgia, Drs. Patrick A. McKee and David G. Sabiston, Jr. of Duke University and Dr. Samuel J. Rapaport of the University of California at San Diego for their participation in this conference. We would acknowledge the valuable assistance of Dr. William Wood, Ms. Betty Nielson, Ms. JoAnn Mueller and Ms. Collete Batten of the Office of Continuing Education in the University of North Carolina School of Medicine.

R.L.L.
W.V.B.
K.G.M.
H.R.R.
April 23, 1980
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HEPARIN

Aspirin, Warfarin, AT-III

Hearing about these has brought me to see,

That when Thrombi and Clots

Tie you in knots,

Only Heparin can set you free.

John B. Graham, M. D.

March 1980

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HEPARIN: AN OVERVIEW

HENRY S. KINGDON
Departments of Medicine and Biochemistry, the Dental Research Center, and the Center for Thrombosis and Hemostasis, University of North Carolina, Chapel Hill, North Carolina 27514 USA

The title of this paper is somewhat of a misnomer, because to attempt to give an overview of heparin that would be comprehensive and touch upon all the topics to be given in this symposium in this short span of time would be impossible. I have, therefore, chosen to dwell for a brief time on the history of the discovery of heparin and its development as a clinically useful anticoagulant, and then to raise a few questions about heparin itself which I hope will be answered in this symposium. Also, I will comment on how we write about research on heparin, and make suggestions that might help us resolve some of the clinical controversies about heparin.

Howell and Holt are widely credited for having discovered heparin in 1918.[1] In their paper wherein the isolation of heparin from liver is described, and its naming takes place, Howell and Holt acknowledge that the material was observed two years earlier by Jay McLean. McLean had accidentally discovered a material which was a powerful anticoagulant while a second year medical student working in Howell's laboratory, working on a project in which he was isolating cephalins from a variety of tissues and attempting to compare their potency in promoting blood coagulation. McLean reported that he had observed a heparophosphatid, which could be separated from cephalin prepared from liver, and which "shows a marked power to inhibit the coagulation".[2]

As has been pointed out by Jaques, however, this simplistic story of the discovery of heparin may not be correct.[3] Jaques points out that Howell changed his extraction procedure in about 1922 from the earlier one utilizing organic solvents to one utilizing aqueous extraction systems. Therefore, what Howell originally called heparin was perhaps indeed a phosphatide, and different from what we call heparin today. Howell himself originally reported heparin was a phosphatide with 5-6% nitrogen and 4-5% phosphorous;[1] he corrected this in 1924 by reporting no phosphorous and the presence of sugar.[4] However, Howell had popularized a name and a concept and the name stuck. His work was probably the rediscovery, purification and characterization of a water extractable anticoagulant material demonstrated and commented on previously by others, among them Morowitz,[5] Doyon et al,[6] and Bayliss.[7]

In view of what we will be discussing in this conference, which will include not only a variety of topics on heparin but also a consideration of the role of antithrombin III in the anticoagulant action of heparin, one other feature of Howell and Holt's original work should be mentioned. The title of their paper was "Two New Factors in Blood Coagulation - Heparin and Proantithrombin".[1] Without going into the data, several comments made in this paper are of interest to our current understanding of the role of antithrombin III and heparin as anticoagulants. "Proantithrombin is activated to antithrombin by heparin". "While heparin itself does not act as an antithrombin, nevertheless it causes a marked increase in the antithrombin of plasma or serum". It appears to me that Howell and Holt had made some very shrewd observations on antithrombin III or heparin cofactor (which they called proantithrombin) and the interaction of this plasma protein with heparin, although the discovery of heparin cofactor is generally attributed over 20 years later to Brinkhous et al.[8] Indeed, Brinkhous mentioned in his discussion the possibility that his heparin cofactor might be the same as Howell's proantithrombin.

Howell had predicted in his Harvey lecture in 1917, that heparin might prove useful in the prevention of thrombosis.[9] However, for nearly 20 years attempts to test this prediction were frustrated by impurities in the heparin preparations available which made them toxic when injected into man and other animals. This is striking in view of the above discussion of the nature of Howell's original heparin, because in the original Howell and Holt paper, heparin had been injected into dogs and shown to have an anticoagulant effect on blood subsequently drawn from the dog, which effect lasted for 3-4 hours. They stated that the injections were very well tolerated. Eventually, through the efforts of a number of workers too numerous to name, including a very prominent group led by Jaques, heparin of sufficient purity for clinical use was finally obtained in a reproducible fashion. Crafoord in 1937 was the first to publish a series of case reports on the clinical use of heparin in man.[10] Crafoord made a particular observation which he considered so important that he stated it twice in the text in italics and as his first conclusion in the summary. "Evidently, both larger and more frequent doses of heparin are required postoperatively in order to obtain the same coagulation reducing effect as in healthy human and animal experimental subjects". This could well have been the forerunner of recent observations that patients with thrombosis require larger doses than normals to achieve equivalent anticoagulant effect.[11]

It is curious that Crafoord's work was an attempt to prevent post-operative thrombosis. This attempt was largely abandoned because of bleeding complications. Now we modern physicians use a new minidose heparin regimen to prevent postoperative thrombosis. "Plus ça change, plus c'est la même chose."[12]

As will be discussed in great detail during the symposium, heparin is partially degraded during preparation from its biological source, and is heterogeneous with regard to a number of structural parameters including molecular size. Before totally abandoning history, let me insert a personal note. I had reason recently to review parts of my own Ph.D. thesis, submitted to Western Reserve University in 1963. In the course of studying the activation of Factor IX by Factor XI_a, I examined the effect of heparin on the reaction, and also the effect of a large series of heparin analogues. These analogues were for the most part dextrans of varying chain length and varying degrees of sulfation. We were uncertain about the mechanistic interpretation of the results due to our protein preparations not being homogeneous, so the results were not published elsewhere. However, the data convincingly supported my conclusion #6 in the summary. "Heparin was seen to be an exception to the rule that at least 20 monosaccharide units and maximal sulfation are necessary for maximum inhibitory activity, demonstrating that variables other than chain length and degree of sulfation are important in determining its potency."[13] Interesting new questions are now being asked regarding exactly what the structural requirements are for the various biological activities which heparin possesses, and whether since the preparations we use are so heterogeneous they could be subfractionated into more specific and better characterized subgroups. I hope answers to some of these questions will be forthcoming at this symposium.

When I was learning to use heparin clinically, which was longer ago than I like to admit, I noticed in many articles that a statement would be made such as "the patient was heparinized" without specifying dose, route of administration, or other important clinical data. This shortcoming in reporting on the clinical use of heparin has now been largely overcome, but certain features of the reporting of heparin usage still trouble me. I am particularly interested in the large number of reports in which heparin is treated by name as if it were a generic drug, and the specific drug company supplying the heparin is not mentioned. I assume in part that this is due to editorial policy with regard to the use of proprietary names for drugs.

However, I believe that this is a less than ideal situation, and may be in part responsible for the fact that some people report heparin-induced thrombocytopenia to be a very common problem,[14] and other people report that it is

rare.[15] Different suppliers of heparin, having made the heparin from different tissue sources, and possibly having materials other than heparin in the preparations, could account for this kind of discrepancy. As an example of something which could be important but which is almost always ignored, I found that one manufacturer supplies heparin in vials with either 1,000 units of heparin per ml or 10,000 units of heparin per ml. Both of these preparations contain 9 mg of benzyl alcohol per ml. Thus, the amount of benzyl alcohol coadministered with the heparin would vary tenfold depending on the strength of the solution chosen from just this single manufacturer. I should comment further that although benzyl alcohol is one of the most commonly used preservatives, it is not present in all heparin preparations and at least historically, other preservatives have been used instead. So it is my firm belief that the company providing the heparin and the strength of solution and any other particulars regarding the heparin preparation should be included in clinical descriptions of the use of heparin, particularly when reporting complications and complication rates. If the policies of clinical journals tend to prevent or discourage this, it is our responsibility to convince our colleagues who are editors that the editorial policy should be changed.

This promises to be an exciting symposium. Dr. Lundblad and the other organizers have assembled an extremely impressive number of experts on the chemistry, physiology, mechanism of action, and clinical use of heparin, which is still the best anticoagulant drug we know. Let us hope that our deliberations at this symposium will allow us to exchange information which will not only be useful to each other in thinking about our own research programs, but which might well lead in time to improvements in the clinical preparations of this extremely useful drug.

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