Anticoagulants and Fibrinolysins

The Ontario Heart Foundation and
The Faculty of Medicine, University of Toronto

INTERNATIONAL SYMPOSIUM

Anticoagulants and Fibrinolysins

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Preface

The prevention of myocardial and cerebral infarction is one of the major problems facing the physician today. Of the measures available for the prevention of thrombosis associated with arterial disease, anticoagulant therapy has seemed the most promising. While the success of anticoagulants in the treatment of venous thrombosis appears to be established, the evidence for benefit in arterial disease is less conclusive. Arterial thrombosis differs from venous thrombosis in that the arterial wall is usually damaged by atheroma and blood flow is rapid and pulsatile. These and other factors may account for some of the difficulty in determining the value of anticoagulant therapy in the prevention of arterial thrombi.

This symposium, held in Toronto in February, 1961, was to a large extent devoted to the problem of arterial thrombosis. Particular attention was given to recent developments in basic knowledge of the etiology and prevention of thrombosis. The practical aspects of anticoagulant therapy were discussed and with this combined approach it was hoped that a better understanding of the usefulness of anticoagulant therapy would be possible. It was decided to include a special section on fibrinolysins because of their potential value in the management of acute thrombosis.

This book contains the papers given at the symposium and the discussions which followed them. The editors would like to thank the participants for their co-operation in forwarding their manuscripts so promptly. We are indebted to the Ontario Heart Foundation for sponsoring the symposium and in particular to Mr. Murray Robertson and his staff for helping in the preparation of the discussions and manuscripts for publication. The publication of this volume is due to the efforts of Mr. R. J. Blacker, President of the Hunter Rose Company Limited and the publisher, The Macmillan Company of Canada Limited.

R. L. MacMillan

J. F. Mustard

Toronto, Ontario

March, 1961

Introduction

The idea that a Conference on Anticoagulants should be held arose in the Medical Committee of the Ontario Heart Foundation some years ago because of conflicting results reported by grantees in different centres. The Chairman at that time, Dr. Hurst Brown, suggested that it would be of value to have a meeting in Toronto of the individuals involved to try to clarify the problem. The original concept soon grew beyond a meeting of local groups because it was felt that with such a limited number of participants the objectives would not be realized, that is, a fuller understanding of the whole problem of Anticoagulant therapy. Discussions with those working in the field soon led to the proposal for a meeting that would be international in scope and would cover all aspects of the problems of blood coagulation and its modification by therapeutic means. A local Committee under the chairmanship of Dr. Fraser Mustard and including Dr. K.G.W. Brown, Dr. R.L. MacMillan, Dr. F.C. Monkhouse and Dr. W. Ford Connell then considered how best this might be arranged and came forward with a plan for a symposium on Anticoagulants and Fibrinolysins. The Board of Directors of the Ontario Heart Foundation supported their recommendations in the belief that a symposium would advance our knowledge in a field that was of vital importance in cardiovascular disease.

The success of the venture is evident in the high calibre of the contributions in this book. What is not evident here but was appreciated by all the participants was the opportunity for individual discussion. These conversations I am sure played just as important a role as the formal presentations in the dissemination of knowledge.

On behalf of the Foundation, I wish to express our appreciation to the contributors and participants in the symposium, to Dr. Mustard and his Committee for the organization of the program, and to Mr. Murray Robertson and his staff for the excellent arrangements for the meeting and the social functions.

John Hamilton

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A Short Essay on the History of Anticoagulants

CHARLES H. BEST (*Toronto*)

One can say quite a lot in twenty minutes but a bit of planning is required to make the most of the opportunity. I have decided to focus your attention for two-thirds of my time on anticoagulants which have, at least experimentally, been shown to prevent thrombosis, but I will begin with the one I first used and which was generally available in physiological laboratories forty years ago, i.e., hirudin.

The date of the first use of venesection in human patients would be difficult to establish. Leeches were employed to replace venesection probably a thousand years ago and their use was widespread until the turn of the century and continues in some countries to this day. My father had them available but I never saw him use them in his medical practice. The druggist or chemist kept leeches in water in glass jars with a bit of sand in the bottom. They were not fed for months at a time but the water was changed. In the 18th century leeches were exported from Bavaria and Bohemia to France by special large "diligences"--the "leech express"! Dr. Erik Jorpes tells us that for many years in Sweden and many other European countries, apothecaries were forced, by law, to keep jars of leeches on their shelves (27). In 1884 John B. Haycraft (18), working in England and also in Strasbourg, reported that blood flow from a leech bite was not easily arrested. He noted that the blood within the body of the leech (Hirudo medicinalis) remained fluid indefinitely and when ejected it did not coagulate. Various experiments were undertaken to determine the source and action of the anticoagulant factor. The conclusion was reached that the leech secretes from its mouth a fluid which destroys the "blood ferment". An extract of leech heads was effective in preventing clotting of dog's or rabbit's blood but not that of Crustacea. It had no action on the curdling of milk, the clotting of myosin or in hastening rigor mortis.

Krüger in Dorpat (34), Dickinson (15), in England, and Erick Schultze (43) of Greifswald in Germany, purified the extract of leech heads and attempted unsuccessfully to isolate the active substance. In 1902 Franz in Jacoby's laboratory in Göttingen (17) attacked the problem. Jacoby was interested in transfusions of blood and suggested to Franz that he should assay the leech extracts under standard conditions on rabbit blood and that he should then use the test as a guide in the purification of the active material. The potency of the leech extract was preserved with thymol and large quantities of stable active material were made available. A method of preparation was evolved which consisted of extracting leech heads in saline for two hours at 60°C. The liquid was centrifuged and the precipitate discarded. The clear supernatant was dried in a desiccator over sulphuric acid. A solution of the dry powder was readily made in distilled water. The active substance was not dialysable and the results of salt precipitations suggested a protein-like structure. In 1903 Professor Jacoby (24) suggested the name "Hirudin".

In 1909 my friend, the late Professor John Mellanby (31), studied the mechanism of action of hirudin and while it is frequently not profitable to attempt the interpretation of older experiments in terms of modern knowledge, it would appear from his results that hirudin contained an antithrombokinase and also an antithrombin. Since Mellanby's thrombin may well have been contaminated with kinase, some of his procedures--even in his careful and experienced hands--gave results which could be interpreted in more than one way. In addition to obtaining strong evidence that hirudin was both an antithrombin and an antikinase, Mellanby showed that fibrinogen removed hirudin from the scene of action. An earlier worker, Bodong (5) had concluded, erroneously it appears, that fibrinogen had been chemically changed by the hirudin so that its potential for fibrin formation was decreased.

Interest in hirudin has continued practically to the present time. In 1929 Waldeschmidt-Leitz, Stadler and Steigerwaldt (49) showed that the anticoagulant action of hirudin was lost during tryptic digestion. In 1957, F. Markwardt (29) of the Pharmacological Institute in Greifswald, isolated hirudin in crystalline form. It is a protein with a low molecular weight of about 16,000 as determined by osmotic pressure measurements. Chromatographic and electrophoretic studies indicate a single substance. Fifteen amino acids have been identified. There are relatively large amounts of cystine, asparagine, and glutamic acid. The substance is a potent antithrombin and has been assigned an activity of 8500 antithrombin units per mg. This would be based on the equivalent number of International Units of thrombin inactivated. The prevention of thrombosis by hirudin has not been studied and the substance has not been used in the treatment of human patients. To complete this picture the exact arrangement of the amino acids in hirudin should be determined, the protein should be synthetized and the actions of the pure product should be subjected to a comprehensive investigation by the most modern and well-established methods.

I must confine my remarks about heparin strictly to the historical aspects because we expect plenty of good-natured differences of opinion about the mechanisms of its various actions and its place in therapeutics or in the prevention of disease. There is controversy about practically all important discoveries. Many people almost made each of these advances. In some cases the claims are false; the scientific world is not convinced by others; exceptionally, the discovery is clear-cut and has been overlooked. I must not consider critically here the contributions of Schmidt in 1892 (39), or that of Doyon in 1912 (16). The latter work, had it been extended, might have anticipated the discovery of heparin by Jay McLean in Howell's laboratory in 1916 (30). There has been some controversy over the question whether the anticoagulant that McLean, Howell and Holt worked with was in fact heparin. It almost certainly was. McLean has described his vicissitudes in 1915 and 1916 when he worked on the "Thromboplastic Action of Cephalin" and "incidentally" but not "accidentally" secured evidence for the presence of a new anticoagulant in extracts of dog's liver (3). In 1918 Howell and Holt (20) wrote: "Attention was first called to this substance (heparin) during some work done in this laboratory by Jay McLean." This was the first public announcement of the discovery of heparin. The name "heparin" was introduced by Howell and Holt. In 1922 (21) and 1925 (22) Howell described the preparation of heparin in more purified form and in 1928 (19) he published a report on its chemical and physiological actions.

In 1928 I made a detailed study of the literature and it was very clear that there was little activity in the heparin field. A potent anticoagulant which could