Preparation of Heparin and Its Use in the First Clinical Cases

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Many of us, who were friends of the late Dr. Jay McLean, had looked forward with great pleasure to seeing him again at this time and to discussing the problems which occupied so much of his attention. We all join Dr. Wright in paying tribute to Dr. McLean, the discoverer of heparin, and to Professor W. H. Howell and his colleagues, who extended this work and focused our attention on many of the most important problems in this field. A number of years ago Dr. McLean wrote to me and asked if we would take the responsibility for his collection of notes and reprints and other documents relating to heparin. I was honoured and extremely pleased to accept this invitation.

It is almost always true that a very careful search of the literature will reveal papers which anticipate, to varying degrees, the discovery of a signal advance in medical or other sciences. In 1912, Doyon published a paper in which he describes an attempt to isolate and characterize an anticoagulant released by the injection of peptone in a dog. This work was interrupted by World War I. There are a number of other intriguing findings in the literature, for example that of Schmidt in 1892, but their significance could only be appreciated after the discovery of heparin by Dr. McLean in 1916.

On November 14, 1940, Dr. Jay McLean wrote me a long letter describing the whole history of his work on heparin, and a great deal about his subsequent researches. I will quote parts of this letter.

You may, however, be interested to know that the first presentation of the anticoagulant at a scientific society was made February 19, 1916, before the Society of the Normal and Pathological Physiology at the University of Pennsylvania. A. N. Richards, the Secretary was then Professor of Pharmacology, and is now Vice-President of the University for the Medical Sciences. These talks were not published although the secretary may have a record in the minutes of the Society... Concerning the lack of articles on heparin in the literature by me, you may be interested in the following. When I wrote the paper on "The thromboplastic action of cephalin," Doctor Howell did not think that I should include anything about the discovery of the anticoagulant. He felt that this should be studied more thoroughly and a paper written about it later. I argued, however, that I had made this finding during that academic year's work in 1915-1916, and felt that it should be included as a record of the work done during that period. I felt this the more strongly because I had already accepted a Fellowship in the Department of Research Medicine at the University of Pennsylvania under Dr. Richard Pearse for the following academic year, 1916-1917 and therefore could not continue the work in Baltimore. He finally agreed to permit its inclusion in the body of the paper.

... At first, Doctor Howell was very skeptical that I had found a true anticoagulant. You know that from my method of preparation, I was using very weak heparin and therefore its anticoagulating action was not noticed with the suddenness and brilliancy of an exploding bomb. Furthermore, you will recall that I was searching for coagulants, not an anticoagulant, and that the end point of my experiments was a clot such as is promptly and solidly formed by cephalin. It was only through very careful records, the systematic saving of the little tubes in which I tested the substances, and then repeating the experiments with the same lot of material and finally making new preparations that I gradually became aware that I had an anticoagulant. Naturally I regard the statements in the literature that I discovered this "accidentally" as not correct. It was discovered "incidentally" in the course of the problem but not "accidentally."

... You will find in the beginning of my laboratory note-book, which I am sending you, the extent of the problem Doctor Howell outlined in his own handwriting, namely, "The preparation of pure cephalin." In looking over this note-book, will you tolerantly excuse its lack of neatness?... As regards the earlier studies with the anti-
coagulant, you might be interested in the following: one author calls my attention to the fourth sentence in the first paragraph of my 1916 paper, which would give one the impression that Doctor Howell suggested that I study cuorin and heparphosphatid for their thromboplastic action. The facts are that the problem Howell originally gave me was simply to make cephalin as pure as possible from the brain and to test each fraction I separated out in the phosphatid group for its thromboplastic action. I finished most of this work between October 15 and January 1916 . . . I first prepared cuorin in January 1916, and it was in January, February and March that I established definitely its anticoagulating action, first of cuorin and then heparphosphatid. It was not until later that Doctor Howell became actively associated in work with the anticoagulant by intravascular injections and mechanism of action in vitro.

I can't think of any other material I have that might be of interest to you. May I, however, offer a suggestion which you may or may not deem worthy of mentioning in your lecture. Doctor Howell has always been perfectly clear and fair in his statements about the discovery of the anticoagulant. As the years go by, more authors credit him with the discovery, apparently disregarding my 1916 publication and the statements he made in his 1917 and 1918 publications. In his Harvey Lecture, he definitely states that this work was done by me, and in his 1918 paper you will note that he says “In the course of his (that is Jay McLean's) work, the anticoagulating action was discovered.” Doctor Howell has always simply stated that he and Holt “first described” heparin.

Dr. McLean attempted several times to return to active experimental work in the heparin field but was engaged in clinical practice. It was apparent from correspondence which I had with Dr. McLean that he had been trying to interest the United States Public Health Service and institutions in various other countries in doing something about the preservation of his notes, reprints, and other heparin memorabilia. He finally decided to send all of these historical documents to us in the Department of Physiology and the Banting and Best Department of Medical Research. The documents are now stored in the Library of The Charles H. Best Institute.

I had many friendly letters from Dr. McLean. He was most generous in his appreciation of the contribution of our group in Toronto. On May 6, 1940, the discoverer of heparin wrote, “I regard you and the work you stimulated in Toronto to have brought about the debut of heparin for clinical use.” My colleagues, Arthur Charles, David Scott, Gordon Murray, Louis Jaques, and T. S. Perrett, deserve a very large share of this praise.

In 1918, Howell and Holt proceeded with the extension of McLean’s work. They state, “Attention was first called to this substance during some work done in this laboratory by Jay McLean,” that is to the substance heparin. Howell and Holt go on to say that they varied the methods in many different ways, and finally selected one which yielded a reliable preparation of heparin. In the copies of these articles in the McLean files, there are many interesting marginal comments; for example, Dr. McLean has pointed out that this description of his work by Professor Howell and Dr. Holt was really the first published announcement of the discovery of heparin. There are many interesting points also in Howell and Holt’s paper. They introduced, for the first time, the word “heparin”; McLean had referred to these compounds carrying the anticoagulant activity as “phosphatids from heart or liver.” They found that heparin could be prepared from lymph glands as well as from heart and liver, as originally shown by McLean. The antagonism between cephalin and heparin on the clotting system was described in the Howell and Holt paper. It has been questioned whether the material that Howell and Holt had was actually heparin, since it was soluble in the crude form in ether. It is now considered that it probably was heparin, since it became insoluble in ether after repeated alcohol precipitations. In 1922 and 1925 Howell described the preparation of heparin in more purified form and in 1928 he published a detailed report on its chemical and physiologic reactions.

In 1924 Mason showed that heparin would prevent the intravascular clot produced in rabbits and dogs by the injection of thromboplastin from tissue extracts. These were true clots and not platelet thrombi. In 1925 my close friend C. I. Reed found that heparin was an effective anticoagulant in dogs and
was well tolerated. In 1927, Shionoya\textsuperscript{10} reported that the administration of heparin did not prevent the agglutination of platelets when blood was made to pass through a colloid tube. Thus it seemed that heparin might be an anticoagulant but not an antithrombotic agent.

Professor Howell undoubtedly anticipated many of the developments which took place in the future. He expressed the hope that heparin would find a suitable application in experimental work and possibly in the therapeutic treatment of disorders of coagulation. Professor Howell thought it not improbable that this substance might be of physiologic significance, and in discussions on coagulation of the blood he often referred to heparin as a "physiological anticoagulant."

While working in Dale's laboratory in London in 1928 I had decided to organize a group, on my return to Toronto, to study the chemistry and physiology of heparin. Later that year, Dr. E. W. McHenry and I, eager to use an effective anticoagulant in our histaminase work, found it possible to prepare active fractions from ox liver by Howell's method. A little later I made a comprehensive study of the literature and it became apparent that very little work indeed was being done in this field. A potent anticoagulant that could be used for long continued administration in animals, was not available. No anticoagulant preparation was safe for clinical work and none was being used. In the Connaught Laboratories I had been intimately concerned with the preparation of insulin and of liver extract for administration to human patients and I visualized a similar advance in the heparin field. Progress was, apparently, also inhibited by the lack of convincing evidence that heparin inhibited platelet agglutination as well as coagulation.

It was obvious that further chemical work on the purification of heparin must precede physiologic and clinical studies. In 1929 I was able to interest a young organic chemist, Mr. Arthur Charles, in this problem, and he made some preliminary studies with me in the Department of Physiology and then joined forces with my colleague of long standing, Dr. D. A. Scott. From that time on the chemical work on heparin was conducted in the Connaught Laboratories, of which I was then an Assistant Director.

On November 10, 1931, I wrote to Professor W. H. Howell:

I would very much appreciate your opinion with regard to several questions in connection with heparin. During the last few years we have been using great amounts of this material in physiologic and bacteriological work. Quite recently, one of the junior members of the Connaught Laboratories, which, as you know, are a department in the University, has interested himself, at my suggestion, in the preparation of heparin from beef liver. He is now in a position to make fairly large amounts of the material which is at least as potent as that distributed by Hynson, Westcott and Dunning. One half gram of this material is being forwarded to you under separate cover. Would you have any objection if this material should be sold by the Connaught Laboratories? (\textit{Note the Connaught Medical Research Laboratories whose objectives are the support of research by the sale of biological products at the lowest possible price.}) I believe that the price would be much more reasonable. As you know, there is a very high tariff on biological products going into the United States so there is very little likelihood of any interference with the American business of Hynson, Westcott and Dunning.

On November 14, 1931, I received the following reply from Professor W. H. Howell in his own handwriting.

I am interested and pleased to know that you have got a usable preparation of heparin out of beef liver. I never could make that source give a decent preparation. As to your selling it, there can be no objection to that, of course. I have feared, at times, that the Hynson, Westcott and Dunning firm would give up its production, as they always claimed that it was a losing proposition to them, so it may be well to have another source. I have been very anxious for them to market a purified heparin, potency 1:50 in 100, but the method I gave to them makes their product too expensive, they think.

The work on heparin in the University of Toronto was the product of activity in three departments—Physiology, the Connaught Laboratories and the Department of Surgery.
Dr. David Scott and Dr. Arthur Charles\textsuperscript{11-15} were extremely successful in their chemical work during the years 1933 to 1936. The most important and novel steps in the preparation and purification of heparin which they introduced were (1) the finding that autolysis of tissue resulted in a much higher yield of heparin, (2) the discovery that beef lung yielded almost as much heparin as liver—this made it possible to use a much cheaper source of raw material, (3) the finding that the destruction of protein by trypsin in the crude protein-heparin complex, was an extremely important factor in the further purification of the anticoagulant, (4) the preparation of a crystalline material as the barium salt—they found that this purified material was of uniform composition and potency. The Danish workers, Schmitz and Fischer,\textsuperscript{16} had isolated in 1933 the anticoagulant material from dog's liver as the barium salt. Neither the barcine salt nor the barium salt lent itself to any large-scale production. Somewhat later Charles and Scott were able to convert the crystalline barium salt of heparin into the sodium salt.

The labels on the bottles of heparin prepared by Hynson, Westcott, and Dunning from dog's liver by Howell's procedure, stated "1 mg. will prevent the coagulation of 5 cc. of cat's blood in the cold." Charles and Scott used this preparation as a reference standard and assigned it a potency of 5 units per mg. In terms of this material the potency of the crystalline barium salt was 110 units per mg, but for simplicity in calculation Charles and Scott decided to assign the figure of 100 units per mg. The material provided for the international standard of heparin was the sodium salt prepared from the crystalline barium salt. The potency of the international standard\textsuperscript{17} of heparin was defined as 130 units per mg., that is, there are 130 arbitrary units of heparin per mg. of the international yardstick. It is calculated that the potency of the international standard is 28 times that of the early Hynson, Westcott, and Dunning preparation. The Connaught Laboratories in Toronto have made two international biological standards—the one for insulin and the one for heparin.

In addition to obtaining heparin in a highly purified form I thought that another point should be settled before the anticoagulant should be submitted for clinical trial. This was the ability or inability of heparin to prevent the agglutination of platelets, which is the first step in the formation of a thrombus as distinguished from a clot.

In 1929, the year after we started our work on heparin, Professor W. E. Gallie, Head of the Department of Surgery in Toronto, nominated Dr. Gordon Murray to collaborate with workers in my department, who were investigating the effects of heparin in the prevention of experimental thrombosis. I was fortunate in having in my department at that time, Dr. T. S. Perrett, a Fellow from the Department of Surgery. I was also fortunate in having a pupil who was taking his doctor's degree in physiology. This student soon became a colleague in the heparin work and a very close friend. He was, as you know, Dr. Louis Jaques, who later became the Head of the Department of Physiology at the University of Saskatchewan and an international authority on many aspects of blood clotting and thrombosis. Dr. Jaques, among the other services which he rendered to our department, sent me one of his own pupils, Dr. Frank Monkhouse, who received his Ph.D. in Physiology from my department in 1952. Dr. Monkhouse is, therefore, my scientific grandson and he, in his turn, has become an authority on different aspects of the great field of blood coagulation and thrombus formation.

The work on the effect of heparin on experimental thrombosis begun in 1929, was pushed forward by Dr. Murray, Dr. Jaques, Dr. Perrett, and myself. We\textsuperscript{18} found that the incidence of obstruction of peripheral veins in dogs by thrombi formed as a result of mechanical or chemical injuries to the intimal surfaces of the blood vessels was definitely decreased when solutions of purified heparin were administered before and for long periods following the injury. These results were obtained in studies of some 300 veins. Thrombi were not observed even after very severe
chemical injury while the animal was well heparinized. We found that the intimal surfaces of veins removed from heparinized animals several days after the injection of heparin had been discontinued appeared, on microscopic examination, to have recovered completely from the injury. The microscopic examinations revealed, in some cases, minute masses of platelets, filling small crevices in the intima. Healing was, however, complete as judged by the absence of thrombus formation after discontinuing the anticoagulant.

The experimental evidence of the prevention of thrombus formation initiated by platelet agglutination, was completely satisfactory before attempts were made to apply solutions of purified heparin to clinical problems.

At various stages in the purification of heparin attempts had been made to use the material as an anticoagulant in transfusing human patients. In 1924 Mason used crude material and obtained reactions which varied from slight chills to severe headache and high fever and nausea. In 1928 Howell used somewhat purer heparin and reported a slight reaction in 2 of 10 transfusions carried out on 6 patients. Godlowski in 1933 reported on the use of heparin in human patients, and although he found low levels of toxicity, the preparation he used was extremely crude and of low potency. In 1936 Hedenius and Wilander studied coagulation times of healthy human subjects. They found that heparin produced no ill effects when the material was given intravenously. This heparin was obtained from Dr. Erich Jorpes and was made by the Charles and Scott procedure.

The work on heparin in Toronto, begun in 1928, proceeded steadily. With each advance in purification we, Murray, Jaques, Perrett and Best, studied the effect on experimental thrombosis and Dr. Gordon Murray made clinical trials beginning in May 1935. When the crystalline sodium salt became available it proved to be safe and effective for the heparinization of patients.

On May 8, 1935, Dr. Jorpes wrote to me from Stockholm in his own hand.

I am sending you a copy of the preliminary report about heparin, and would like to use this opportunity to thank you for all the hospitality shown to me and to Mr. Bjurling during our visit in Toronto in 1929. We have greatly benefited from your experience in the manufacture of insulin.

The heparin work has been a very hard task. For a very long time I believed that my preparations were only impurities as compared with those of Charles and Scott. I greatly admire their working capacity. They have opened this field, which before them was quite hopeless.


I have been interested in some physiological work on heparin recently; as a matter of fact, we have been administering some to human subjects. I hope that we will see you at the Physiological Congress in Russia this summer.

Up to the time of this letter there had been no published reference to the use of highly purified heparin in clinical cases but as Dr. Jorpes has written, the idea of using heparin to prevent the formation of thrombi was in the minds of all who came in close touch with the problem. Its realization merely depended on the availability of a satisfactory preparation of heparin. The clinical problem was attacked in Toronto and in Stockholm, as soon as pure heparin was obtainable. The studies by Crafoord and later by other workers in Sweden, were proceeding at the same time as those of Dr. Gordon Murray in the Toronto General Hospital. The results obtained clearly indicated that certain types of clinical thrombosis could be prevented by the treatment with purified heparin. These findings were made possible by the preparation of pure heparin from beef liver or lung. The word pure is used here to indicate a uniform preparation, of standard potency, and free from toxic components rather than in the true chemical sense.

Dr. Jorpes and his colleagues have made a very large number of fine contributions to the heparin field. The cellular origin of the anticoagulant, the chemistry, the mechanism of action, the clinical use in a great variety of conditions, and many other subjects have been illuminated by the work of this group, which
is well summarized by Dr. Jorpes\textsuperscript{24, 25} in his monographs. I have had the pleasure of knowing a number of the Swedish "anticoagulationists" in addition to Dr. Jorpes. Dr. Per Hedenius and the late Dr. Hjalmar Holmgren have been particularly close friends.

Our present knowledge of the chemistry of heparin has been summarized by Dr. Arthur Charles\textsuperscript{26} as follows: "Heparin is a complex polysaccharide. The carbohydrate moieties are glucuronic acid and glucosamine which are present in the molecular ratio of 1:1. The carbohydrate is highly sulphated. The amino group is not free and does not appear to be acetylated as in mucoitin or chondroitin sulphate. Evidence has been presented which indicates that the nitrogen is sulphated."

The availability of well standardized heparin not only made possible the clinical work but a very great deal of experimental study. Without this potent heparin the exchange transfusion experiments, carried out by Thalhimer, Solandt, and myself,\textsuperscript{28} would not have been possible. The dramatic use of the artificial kidney by Kolff and Berk\textsuperscript{29, 30} in Holland and by Dr. Gordon Murray in Toronto,\textsuperscript{31, 32} also depended on purified heparin. I will not attempt to make a complete list of the advances which the availability of potent purified heparin has facilitated.

There will obviously not be time to follow in detail the many lines of interest which developed in the middle 1930's. Members of our own group were interested in the source of heparin and its appearance in blood in peptone and anaphylactic shock. The work on Witte's peptone goes back to the publication of Schmidt-Mülheim\textsuperscript{33} in 1880, when it was shown that injection of the material in dogs produced shock and incoagulability of the blood. In 1909 Biedl and Kraus\textsuperscript{34} found that the blood failed to clot in anaphylactic shock. Professor Howell\textsuperscript{16} in 1925 and Quick\textsuperscript{35} in 1933 had obtained anticoagulant preparations from dog's blood after injection of peptone. The subject was further advanced by Wilander\textsuperscript{36} in 1939, who isolated heparin in amounts sufficient to explain the coagulation deficiency. Waters, Markowitz, and Jaques,\textsuperscript{37} in our laboratory, showed in 1938, that the incoagulability of the blood, both in peptone shock and anaphylactic shock in dogs, was completely inhibited by protamine. The dramatic neutralization of the effect of heparin by protamine had been shown by Chargaff and Olson\textsuperscript{38} in 1937. In 1940 Jaques and Waters\textsuperscript{39, 40} isolated a barium salt of pure heparin from the blood of sensitized dogs given serum albumin.

Another point of interest in our laboratory was the enzymatic destruction of heparin by material prepared from rabbit's liver. This was carried out by Jaques\textsuperscript{41} in 1940 and he suggested the name "heparinase" for this system. The use of silicone in preventing clotting was introduced by Jaques, Fidlar, Feldsted, and Macdonald\textsuperscript{42} in my laboratory in 1946. This was a great improvement over vaseline or paraffin, which, of course, had been used ever since the work of Freund\textsuperscript{43} in 1888 and of Bordet and Gengou\textsuperscript{44} in 1901. A very great many experiments have been facilitated by the use of silicone coating of glass tubes, needles, and other apparatus.

The experimental work which D. Y. Solandt, Reginald Nassim, and I did\textsuperscript{45} on the prevention of coronary thrombosis and intramural thrombosis in dogs by the administration of heparin, fascinated us until problems of military medicine diverted our attention in 1939. Dr. Solandt and I\textsuperscript{46} described a method by which gradual occlusion of coronary arteries by thrombus formation may be produced in experimental animals. The thrombus formation and the resulting cardiac infarction were in a very large part prevented by the administration of adequate amounts of highly purified heparin. In discussing the possible clinical application of our findings I\textsuperscript{47} wrote, in 1938, "If the clinical investigation of cardiac cases should be initiated, the necessity for studying very large numbers and of heparinizing only alternate cases is obvious."

In the investigation which Dr. Solandt and I made with Dr. Nassim, we evolved a method by which cardiac mural thrombi could be produced in animals. These thrombi were formed very rapidly and there was a very dramatic and extensive fall in blood platelets during this interval. The formation of the mural
thrombi could be completely prevented by the administration of adequate amounts of highly purified heparin. We wrote that at that time, in 1939, "Since over ten per cent of the deaths associated with coronary thrombosis in man are caused by embolic sequels of mural thrombus formation, a clinical trial of heparin is indicated."

There was an attempt, in Toronto, to apply some of these results, but no comprehensive investigation was found to be possible at that time. In 1948, Wright, Marple, and Beck wrote, "The possibility of preventing the extension of coronary thromboses and the development of mural thrombi in the presence of myocardial infarction by the use of anticoagulants was suggested by Solandt, Nassim and Best in 1938... Their observations were not applied to human beings on any significant scale because of the difficulties and the risk felt to be inherent in the use of heparin clinically."

When purified heparin became available in Toronto requests for this material for experimental and clinical use came from many parts of the world. One of the earliest was from Dr. Leo Mayer who wrote on December 21, 1938, "Dr. Irving Wright of the New York Post Graduate Hospital has suggested the advisability of using heparin in this case." The patient was Mr. Arthur Schulte. I remember sending heparin to Dr. I. S. Ravdin of Philadelphia who needed it for the postoperative treatment of a brilliant young doctor who had a saddle embolus at the bifurcation of his aorta. Many surgeons and physicians came to Toronto to discuss the clinical problems with Dr. Murray, or physiologic or chemical matters with our group. I remember many of these men vividly—Dr. Essex and Dr. Priestley of the Mayo Clinic and Dr. Lahey of Boston were among those who came from this country.

The interest in heparin continues to grow. Dr. Jay McLean was undoubtedly fascinated by the effect of heparin on the clearing of lipemic plasma, first demonstrated by Hahn, and by the great volume of recent literature on the effects of the anticoagulant on fat mobilization.

Heparin has thus already removed many barriers to the free flow of knowledge but we are still in the early stages of appreciation of its physiologic and clinical significance.

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