Heparin: A Mucopolysaccharide and an Active Antithrombotic Drug

By J. Erik Jorpes, M.D.

HEPARIN, like so many other biological substances, was discovered incidentally. William H. Howell, professor of physiology at Johns Hopkins University, Baltimore, was in 1916 trying to isolate a thromboplastin, an accelerator of the coagulation of the blood, from the phosphatide fraction of the liver and the heart. His co-worker, Jay McLean, found instead a substance, later called heparin, which retarded the coagulation of blood. Also unexpected was the finding made almost 20 years later in 1935 that heparin is a mucopolysaccharide esterified with sulfuric acid to a quite extraordinary degree.

The Chemical Nature of Heparin

The closest chemical neighbor of heparin, chondroitin sulfuric acid, was in the 1920's in spite of its interesting chemical composition still not easily accessible. It shared with the nucleic acids the property of being a macromolecular ester of a strong mineral acid, in this case sulfuric acid. In 1928, I succeeded in obtaining a protein-free chondroitin sulfuric acid having an almost theoretical content of ester sulfate by adsorbing the proteins on kaolin. This preparation was then used in our laboratory as a reference substance for checking the methods used for the quantitative analysis of uronic acids.

Among other natural products analyzed for uronic acids we also included in 1934 the heparin, claimed by Howell to give a positive color reaction for uronic acid. Heparin had been isolated in 1933 in a highly purified state by Charles and Scott of Toronto. In fact, the Tollens-Lefèvre technic showed a considerable content of uronic acid in heparin, a content which increased with increasing anticoagulant activity, making up almost 20 per cent of the dry substance of the purest heparin preparations.

The Tollens-Lefèvre technic is quite reliable and is easy to perform. The same could not be said about the methods applied for the biological assay of heparin. Cats were not so easy to get, and it was difficult to find the operating room and the assistants needed. An easier solution was then found. Series of test tubes containing a glass bead and serial dilutions of heparin solutions were filled early in the morning with fresh ox blood at the slaughter house, and readings of the coagulation times were made at intervals during the day. In 1 day several heparin samples of unknown strength could be compared with a standard heparin practically without any cost. This technic opened the field for further experimentation on a larger scale.

The purified heparin samples were found to contain large quantities of a hexosamine, which was later shown to be glucosamine, amounting to one mole of hexosamine per mole of uronic acid. At that time, 1933, Elson and Morgan had improved the Zuckerkandl-Klebermass method of 1931 for the quantitative analysis of hexosamines. We also believed that we had found acetic acid as a third component like that of the chondroitin sulfuric acid, but this assumption, based on faulty technic, soon proved to be erroneous.

The organic skeleton of heparin thus showed similarity to that of the chondroitin sulfuric acid. This acid, however, has no anticoagulant activity. The analysis of the ash, which amounted to not less than 25 to 40 per cent of the different preparations of the purified heparin, then gave the key to the problem. It was found to consist of sulfates exclusively. The sulfate was precipitable with barium chloride after the heparin was first hydrolyzed with mineral acid. This indicated an ester linkage

From the Chemistry Department II, Karolinska Institutet, Stockholm, Sweden.

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of the sulfates similar to that in the chondroitin sulfuric acid. Now, when the sulfur analysis indicated a sulfate content of about 40 per cent in the purified heparin, 2½ times more than in chondroitin sulfuric acid, difficulties arose in convincing workers in this field that such compounds could exist in nature. It was an easy matter, however, to induce anticoagulant activity in ordinary polysaccharides by treating them with chlorosulfonic acid.

At almost the same time, 1938, however, Soda and Egami in Japan found in the visceral hump of a mollusca, Charonia lampas, a similar compound, a polysaccharide containing 15 per cent of sulfur as ester sulfate. Many years later, 1947 to 1950, Vasseur in Sweden showed that the mucous layer surrounding the sea urchin eggs contains plenty of polysaccharide polysulfuric esters with an ester sulfate content of about the same order as that of heparin. The organic skeleton of these compounds is built up of hexoses and methylpentoses, varying for different species.

It was thus evident that heparin belonged to a group of natural substances called mucopolysaccharides containing a hexosamine and a uronic acid, and that polysaccharides likewise highly esterified with sulfuric acid can be synthesized even by invertebrate animals. One detail, however, in the chemical structure of heparin is unique, the sulfamide or amidosulfuric acid group. In heparin, 1 sulfuric acid group is linked to the amino group of the glucosamine, as suggested by Masamune in Japan in 1940 and by Wolfrom in the U.S.A. in 1943 and finally demonstrated by our group in 1949.

It may not be quite out of place to point out that a neglected elementary analysis, in this case the sulfur analysis, evidently delayed the elucidation of the chemical nature of heparin, a by no means uncommon fatality. Liebig himself missed the phosphoric acid in his inosinic acid, the first nucleic acid described. Fifty years later it was shown to be a nucleotide. The sulfur content of taurine was likewise overlooked by Pelouze and Dumas in 1838. Also the sulfur of the thio-methylpentose of the yeast was missed, and made the sugar difficult to identify until the sulfur was found by Zuzuki and Mori in 1926. Two oxygen atoms put into the place of 1 sulfur atom made the elementary analysis fit. Even the sulfur of vitamin B₁ escaped detection when the newly crystallized vitamin was analyzed by Jensen in 1926.

It must be pointed out however that Howell, although being a physiologist, did not miss the sulfur in the ash after igniting the heparin preparations, but he found it quite natural to speak about the ash as a contamination of the samples.

**Heparin and the Mast Cells of Ehrlich**

In 1936 the Stockholm group found that the heparin is produced by the mast cells of Ehrlich, a discovery which gave rise to an overwhelming literature dealing with these cells. Lison's finding of 1933 that the purple metachromatic staining of cartilage and mucous membranes given by toluidine blue is due to the ester sulfate group of chondroitin sulfuric acid caused me to apply the reaction to heparin. With heparin it was 100 times stronger than with the chondroitin sulfuric acid.

A stimulating observation was made when swine thoracic aorta was immersed for a short while in a 0.01 pro mille solution of toluidine blue. The most brilliant color in purple lilac was developed on the inside of the aortic intima. We immediately anticipated that the heparin should form a superficial layer inside the aortic wall which with its ionized ester sulfate groups could exert some kind of repellent action. Unfortunately for the hypothesis, the metachromatic staining was due to the chondroitin sulfuric acid present in the intima.

The location of the heparin in the mast cells of Ehrlich was made by Hjalmar Holmgren, assistant at the Histology Department of our Institute. When we asked him to locate the heparin in the body by means of the metachromatic reaction, he could the next morning inform us that the mast cells of Ehrlich, a kind of cells quite foreign to us at the Chemistry Department, were carrying the heparin
in their granules. The quantitative analysis performed by Wilander also showed 10 times more heparin in the capsula Glissonii, the liver capsule, which is extremely rich in mast cells, than in the liver parenchyma itself. Since that time much, possibly too much, has been written about these cells and many more functions have been assigned to them than the order of Nature reasonably can have bestowed upon them.

**Heparin as an Antithrombotic Drug**

Clarence Crafoord, the well-known Swedish thoracic surgeon, had drawn attention to himself already as a very young physician through his numerous pulmonary embolectomies (between 20 and 30). What would be more natural in a case like his than to go to a biochemist and ask him to get out the heparin of Howell to be tried as a prophylactic against pulmonary embolism. This was in fact what Crafoord did in 1929. The only answer we could give him was unfortunately, "Non possumus."

In 1935 we instead could approach him at the Sabbatsberg Hospital and ask him to try out our heparin preparations clinically. In the meantime Hedenius and Wilander had performed the first intravenous heparinization on themselves outside of the hospital. Their finding that 100 mg. or more of heparin are needed for heparinizing a human being for a few hours caused at first an almost desperate feeling. It seemed to be impossible to get sufficient material for a heparinization on a large scale. All the work on the chemistry of heparin had been performed on 6 Gm. We could not anticipate at that time that we in cooperation with a pharmaceutical house, Vitrum AB, Stockholm, within a few years should be able to produce 1 Kg. or more a week of the new substance.

Crafoord immediately started a series of experiments heparinizing patients postoperatively. Many an older colleague shook his head and expressed his dislike of such experiments, in which the patients were "made hemophiliacs" for a time. Crafoord, anyhow, fulfilled his intentions and treated 325 pa-

-tients with heparin postoperatively. His colleague, Per Wetterdal, of the Gynecology Department of the same hospital, contributed another 231 cases and Leissner of the Maternity Clinic of the University of Lund heparinized 309 patients post partum. In total, about 800 cases were thus given heparin after operation or childbirth. A high frequency of thrombosis, at least 3 to 4 per cent and possibly still higher, was expected in Crafoord's series if untreated consisting only of patients over 35 to 40 years of age and with operations known to be followed by a relatively high percentage of thromboembolic complications. Practically no incident of that kind occurred. Although Wetterdal's and Leissner's series comprised only selected cases expected to give a high frequency of thromboembolism, no complications were observed during the first 10 to 15 days after operation or delivery. Among the 657 (325 + 140 + 192) cases receiving 250 mg. or more of heparin daily for 5 to 10 days no signs of thrombosis occurred.

Similar experiments were at the same time going on in Toronto, Canada. In order to demonstrate the usefulness of heparin in inhibiting thrombosis a series of animal experiments, initiated in 1932 in the Department of Surgery of the Toronto General Hospital, was performed in close conjunction with the chemical work on heparin at the Connaught Laboratories of the University of Toronto. These experiments were reported by Murray, Jaques, Perrett, and Best in 1936 and 1937. In 1938 Solandt and Best published their well-known paper about the dissolution of fresh thrombi in the coronary arteries of dogs by perfusing the vessels with a dilute heparin solution. Gordon Murray at the Toronto General Hospital contributed at first 260 cases and then a total of 400 cases treated prophylactically with heparin. He reported results equally as good as the Swedish group.

Thus the postoperative course of the more than 500 carefully controlled cases of Crafoord and of Wetterdal, supplemented by the 309 cases of Leissner and the 260 cases of Murray and MacKenzie, a total of 1,151 patients, seemed to prove that heparin, if rou-
tinely used over a sufficient length of time, gives an almost complete protection against thromboembolic complications after surgical operations and childbirth.

Anticoagulant Therapy in Thrombosis

As a result of the lively interest in heparin following Crafoord's first publication in the spring of 1937 on prophylactic heparin treatment in man and our discovery of the connection between heparin and the mast cells of the same year, a physician in Stockholm, Holmin, later in the year tried the new remedy in a case of fresh acute thrombosis in the central retinal vein in a young person. Well aware of the hopeless prognosis, he gave a tentative dose of pure heparin intravenously 3 to 4 times daily over a period of 10 days beginning on the third day of the illness.

Ploman describes the course of this case as quite unusual, for the patient regained a visual acuity of 0.9 in 9 days. In a second case, described by Boström and William-Olsson, where the lesion was 1 month old, visual acuity rose from 0.1 to 0.4 in 5 days and later to 0.6. The unusual course of these 2 consecutive cases made these ophthalmologists inclined to ascribe the result to the treatment with heparin.

In the same year Magnusson (1938) used heparin successfully in a case of thrombosis of the posterior inferior cerebellar artery, Wallenberg's syndrome, a disease in which a regression is unusual. In 1938, Murray and Best reported 28 cases of spontaneous thrombophlebitis and 7 cases of pulmonary embolism treated with heparin. All the cases of embolism showed rapid clinical improvement, and the 28 cases of spontaneous thrombophlebitis showed no evidence of embolism, and the clinical signs and symptoms, pain, swelling, tenderness and fever, appeared to show more rapid improvement than in a control group.

In his second paper Crafoord (1939) stated that he had given heparin to 20 patients with manifest, thromboembolic complications. In some of these both the general and local symptoms receded strikingly rapidly.

In Sweden, Magnusson (1940) administered heparin to a woman with severe pulmonary embolism and thrombosis in both legs, complicating a postpartum scarlatina. The temperature became normal in a few days and the patient, who had been ill for 6 weeks with repeated thromboembolic recurrences and was very emaciated, recovered.

After these most dramatic and very convincing preliminary experiences with heparin discussion of the anticoagulant therapy was broadened and taken up on a larger scale in different parts of the world. It was quite evident that there could be no question about a general prophylactic heparinization. Early rising after operations and childbirth makes such a measure superfluous except in some cases with a pronounced tendency to thrombosis. Then Karl Paul Link's work, leading to the discovery and synthesis of Dicumarol, broadened the field in a highly desirable way by making a prolonged anticoagulant therapy possible. The easily accessible oral drug also proved a prerequisite for long-term prophylactic treatment.

It may be added here that active movements and early rising from the bed are now generally prescribed and strictly applied in most countries. The question then arises to what extent the beneficial effects of the anticoagulant therapy observed are due to the anticoaguants or to the movement therapy. In fact the most critical observers in Denmark, the country of Hans Christian Andersen, have spoken about the Emperor’s New Clothes in thinking of the advocates of the anticoagulant therapy, a very sound criticism indeed. Without active movements and early rising from the bed the effect of the anticoagulant treatment would certainly not have been so good.

It is in fact impossible to evaluate these experiences correctly. The fact remains, however, that thrombosis can be prevented through prophylactic heparinization. As to anticoagulant therapy in thrombosis, it must also be kept in mind that prior to this therapy thrombosis of the veins of the legs used to be so painful and the legs so swollen that there could be no thought of active movements and early rising. Anybody treating a severe leg
thrombosis or a pulmonary embolism with heparin will soon be impressed by the amelioration of the pain and of the feeling of oppression, and by the disappearance of the swelling of the leg. It would also have been deplorable if the medical profession had been unable to detect the value of the movement therapy without the stimulant of the new anticoagulant therapy. Until the most recent years thromboembolic patients were kept in bed for 6 to 8 weeks.

Under the influence of this discussion a gynecologist at one of our university clinics decided to treat a series of cases of acute thrombosis without heparin. The first patient was sent home and instructed to move around as much as possible. A few days later she came back with a florid phlegmasia alba dolens. His series comprised only this case.

In the beginning of the 1940's the large-scale clinical experiments with the anticoagulant therapy had begun in Sweden (Hellsten, Bauer, Zilliacus), in the U.S.A. (Allen, E. V. and Barker, N. W., Wright, I. and co-workers, de Takats, G.), and in Switzerland (Merz, R. W.). Heparin and Dicumarol thereby left their cradle, the laboratories of physiologic and organic chemistry. They both proved valuable enough to keep their position in a world where new therapeutic products flourish and disappear in a continuous stream. An almost immense literature already tells their story.

In speaking about the cradle of heparin it is not out of place to mention that the writer had the pleasure of being able to inform William II. Howell of Johns Hopkins University during his last years about the successful progress of the anticoagulant therapy in this part of the world. Needless to say, these reports were welcome. They told him that something of permanent value will remain as a result of his contributions to physiology. They might also to some extent have enlightened those dark days during the war, as that of April 11, 1942, when he wrote, "When this killing and shooting is all over I fear that this world will not be such a pleasant place to live in as it was in my youth—and I shall have no great regrets in leaving it, although I would dearly love to know what steps will be taken to assure a permanent peace."
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