100ème ANNIVERSAIRE DE LA DECOUVERTE DE L'HEPARINE

By way of introduction, a tribute to Jean Choay- Pierre Sinaÿ (UPMC, Paris, France)

Dear Friends and Colleagues, Ladies and Gentlemen,

It is a real pleasure for me to open this morning session. We are here today to celebrate the anniversary of an old lady, the 100 years of heparin. Does it mean that this distinguished polysaccharide was discovered ex abrupto in 1916? Absolutely not. What happened then in 1916, when the First World War was raging on in Europe? Back in 1897, a renowned US physiologist, William Henry Howell, edited a book entitled an American Text-book of Physiology, a classic in the field. In 1914 a young medical student, Jay McLean, discovered this textbook and through the reading became fascinated by the research possibilities it offered. So fascinated that he decided to join Howell's laboratory at Johns Hopkins Medical Center in Baltimore on his own spare money, which implies for a limited period of time. He had, as he reported much later in his Memories, the dogged determination to know whether he would be really capable to accomplish something by his own ability. Based on that we can say, in a way, that Howell's textbook proved historically crucial to the discovery of heparin, as it would led Jay McLean to seek out Howell's laboratory. Dr. Howell was an established master in the field of blood coagulation. In 1912 he demonstrated that cephalin, a member of the phospholipid family, is a thromboplastic substance and, in 1915, when McLean arrived in Baltimore, he worked, on Howell's suggestion, on this finding, to determine whether a similar tromboplastic activity would also be exhibited by other related phosphatides, isolated and purified from various organs. He first quickly confirmed that thoroughly purified cephalin from brain has pronounced tromboplastic activity. Next, McLean concentrated on two phospholipids previously isolated by two German scientists. The corresponding publications were written in German language in Zeitschrift für Physiologische Chemie. In order to repeat the described isolation procedure, he very quickly learned enough German language. This is a further demonstration of his determination. He incidentally discovered a fraction, very soluble in ether, yes, hear me, very soluble in ether, which had no thromboplastic action, but in stark contrast showed a marked power to inhibit coagulation. He named the extract heparphosphatid, that is to say a phosphatide extracted from liver. Nothing to do with a carbohydrate. Howell, clouded by coagulation, did not think that McLean should include in his

paper about this serendipitous discovery of this anticoagulant. McLean argued, has reported in his Memories, that he had made his finding during the Academic Year 1915-1916 and that it should be included as a record of the work done during that period. Howell finally agreed to permit a tiny inclusion in the body of the paper devoted to coagulation. In 1916, the paper appeared in the American Journal of Physiology, entitled « The Thromboplastic action of Cephalin ». It is a paper on coagulation. We have to read it very carefully to detect a reference to the anticoagulant. Howell was indeed originally very sceptical that McLean had found a true anticoagulant but, as soon as McLean left his laboratory, without spare money anymore, he switched from his own well-anchored field of coagulation to the anticoagulation of his alumnus and, in 1918, he published, with a new student named Holt, a paper entitled « Two new factors in blood coagulation: heparin and pro-antithrombin ». The word heparin was coined. McLean's product in 1916, Howell and Holt two products in 1918 are extracted in ether. They are phospholipids, nothing to do with the polysaccharide heparin, which is totally insoluble in ether. 1918 version of heparin is not heparin! In 1916, we had neither the name nor the product, just a mode of action. In 1918, we had the name, but again not the product.

But the whole lot toppled over in 1922 from lipids to sugars. What happened then? Howell and Holt modified completely the extraction procedure, using water in alkaline conditions and this time for sure they isolated the polysaccharide heparin. This new protocol was presented at the annual meeting of the American Physiological Society in 1922. In 1926, Howell presented further refinements to the protocol and, ultimately, here we are, Howell, in 1928, in the first recapitulative article on heparin, published in the Bulletin of Johns Hopkins Hospital, totally confirmed that the anticoagulant was of glucidic nature and contained, neutralized as salts, sulphates and uronic acids. These are facts, from the literature.

What is a discovery? It is almost always true that a very careful search in the literature will reveal papers which anticipate, yes which anticipate, the discovery of a signal advance in medical or other sciences. With this in mind, it is thus not unfair to say that McLean's serendipitous finding in 1916 changed the focus of Howell's research, pointing him in the right direction, culminating with the aqueous alkaline extraction and entry into the carbohydrate world. We can speak of the prodigious decade 1916-1926, triggered by McLean's observation, Howell being the official discoverer of the carbohydrate.

By the way, to end up with this baltimorian story and to move, in the remaining time, to my announced tribute, there is an opened question: what pushed Howell to change the extraction procedure? Intuition, or something else? In 1912, Doyon, a medical researcher at the Faculty of Medicine of Lyon in France, published, in French language, in Comptes Rendus de la Société Biologique, the isolation of an anticoagulant from various organs, using an alkaline aqueous solution. This is exactly the protocol used and published ten years later by Howell, without any reference to Doyon's work. It is not unlikely that Doyon's anticoagulant was the polysaccharide heparin. The First World War interrupted this research in France. This is life.

Johns Hopkins Hospital in Baltimore is the birthplace of heparin and Howell the driving force of its discovery. About fifty years later, another place in the world witnessed the birth of the active site of heparin, a major breakthrough in heparinology you are probably familiar with in this audience. A place and a driving force. The place is Institut Choay and the driving force is Jean Choay.

Who is Jean Choay, born when William Howell, across the Atlantic Ocean, discovered the real carbohydrate nature of heparin. His grandfather Eugène Choay had founded the family Choay Company, Jean Choay joined in the 50's, not before he had shown a keen interest in the Classics, with a master degree in Philosophy and a dissertation in Epistemology. This explains a good many things. His achievements mainly emerged from his foresight in founding in 1969 the Institut Choay, a place for intellectual stimulation and productivity. In his book les deux pieds, les deux mains dans le médicament, Pierre Simon wrote, I leave it in French: Jean Choay, un vrai savant, peut-être un peu trop, au fond de lui-même plus passionné de découvertes scientifiques que de nouveaux médicaments, plus sensible aux honneurs académiques qu'à une réussite industrielle. Un charme et une intelligence qui réussissaient à faire oublier une disgrâce physique majeure.

I met Jean Choay for the first time during a lunch at Orsay University, organized by the eminent biochemist Edgar Lederer. He told me: Mister Choay would like to propose something to you. The scientific link between Edgar Lederer, Jean Choay and myself was muramic acid, a basic and typical monosaccharide component of bacterial cell wall. Edgar Lederer, at the Institut de Biochimie d'Orsay, was working on the chemical elucidation of the minimal structural requirement for immunoadjuvant activity, which is a derivative of muramic acid. Jean Choay at Choay Institute was interested in the possible medical

applications. Myself in Orleans with Claude Merser could easily achieve the chemical synthesis of Muramyldipeptide (MDP), which indeed unequivocally proved to be the minimal structural requirement for the adjuvant activity of bacterial peptidoglycan derivatives. A piece of work of medical importance, combining meticulous structural studies, based on chemical and enzymatic degradations, with at the end chemical synthesis tackling the real active site and offering the possibility of a potential drug development. What happened then during Pear and Cheese this day at Orsay? I was fully unaware of Choay's beautiful and classical work on heparin fragmentation and fractionation and of his search for the minimal structural requirement for anticoagulant activity. So, in analogy with MDP, he proposed me to collaborate with Institut Choay on the chemical synthesis of the real active site of heparin. I resisted first, pentasaccharide is not a child's play, as MDP was. No, it is much too complicated. But the radiant personality of Jean Choay, his communicative scientific enthusiasm convinced me to try hard. Maurice Petitou, a research student in my group in Orleans, joined Institut Choay, 10 rue Morel in Montrouge with a fascinating PhD project: the synthesis of the active site of heparin. Jean-Claude Lormeau was in charge of biological activity studies and a fruitful collaboration was established with the Ronzoni Institute in Milano for NMR structural investigations of heparin fragments. You know what happened. In 1983 was published in Biochemical and Biophysical Communications a paper entitled: Structural activity relationship in heparin: a synthetic pentasaccharide with high-affinity for antithrombin III and eliciting high anti-factor Xa activity.

It was a major breakthrough in heparinology for the Choay team and associated partners in Milano and Orleans, which introduced synthetic heparin oligosaccharides as a potential new class of antithrombic drugs. The road to Arixtra was opened. It has been a great chance for me to keep close to Jean, an eminent scientist and a man of immense culture.

Thank you.