Anniversary Issue Contribution

The discovery of factor X: A personal reminiscence

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When I chose to become a physician at age 15, I had in mind to practice internal medicine in a medium-sized city not too far from Zurich, Switzerland. However, fate decided otherwise. After my graduation from medical school I was searching hard to find a place as an intern and resident but realized that this was hardly possible in the city of Zurich. At that time about three quarters of all interns and residents were unpaid, and even these volunteer positions were extremely hard to get. I went to a smaller hospital in the vicinity of Zurich where Dr. Fritz Koller (Fig. 1) was head of the internal medicine department. Asking him for an internship, I was told that there was a long waiting list to obtain an unpaid internship, but – "Do you have the scientific fire for research" he asked me unexpectedly. I was baffled at this question but Dr. Koller explained to me that there was a position available at his haemostasis research laboratory at the Department of Medicine of the University Hospital in Zurich. The Emil Barrel Foundation sponsored this position with $125/month. I started a few days later and was told to find out more about the presumptive factor X that had been postulated by François Duckert, Paul Flückiger, Martin Matter and Fritz Koller (1).

The fifties were an exciting time in the field of blood coagulation. In the forties factor V/labile factor/accelerin had just been discovered. In 1949 Alexander et al. postulated, on the basis of quite inconclusive data, the existence of a further coagulation factor, termed serum prothrombin conversion accelerator (SPCA) (2). A first case of a congenital deficiency of this factor was described in 1951 (3). In the same year further publications confirmed the existence of a stable factor, present in plasma (Owen and Bollman), called proconvertin by Owren and factor VII by Koller et al. (4, 5). The latter article became a citation classic and has now been cited 477 times (6). Paul Owren was possibly aware of the forthcoming article by Koller et al., when in spring 1951, he published a one-page description of proconvertin in the Scandinavian Journal of Clinical and Laboratory Investigation (4), followed a few months later by more complete descriptions (7, 8).

In 1952 plasma thromboplastin component (PTC)/ Christmas factor (later on called factor IX) was independently discovered by Aggeler et al. in San Francisco and by Biggs et al. in Oxford. The thromboplastin generation test developed by Biggs, Douglas and MacFarlane in 1950 was to become a very important tool in the differentiation of factor VII from factor X, of factor VIII (antihaemophilic globulin) from factor IX, and in the discovery of plasma thromboplastin antecedent (PTA, factor XI) in 1953 and Hageman factor (factor XII) in 1955 (9).

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The field of coagulation seemed to explode in these years, and very often discoveries of new factors were made independently by researchers in Europe and the United States, resulting in a profusion of different names. Under the leadership of Irving S. Wright, an international Committee for the Standardization of the Nomenclature of Blood Clotting Factors was created at a meeting in Basel, Switzerland in 1954. In 1958 the committee met in Montreux where the Roman numeral designation of clotting factors was proposed by Koller. Many reservations to this proposal were voiced (10–12), but finally it was adopted and the Stuart-Prower factor became officially factor X. Gradually the tasks of this committee expanded and included standardization issues. The name was changed to International Committee on Thrombosis and Haemostasis (ICTH). Fritz Koller was its first secretary general from 1960 to 1962.

Back to my task. Under the guidance of François Duckert (Fig. 2), a biochemist, I had to prepare protein fractions devoid of factor X and to test them using the thromboplastin generation test of Biggs and collaborators. I must have performed thousands of these tests, but the presumptive factor X remained an unsolved enigma. I remember how I was labouring to produce fractions

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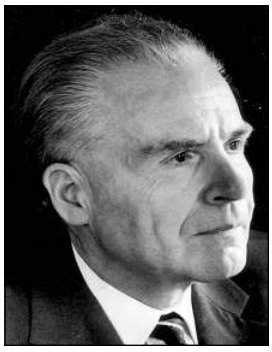

Figure 1: Fritz Koller (photo courtesy of ISTH).

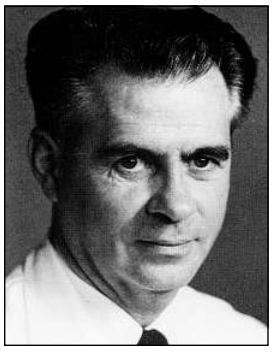

Figure 2: François Duckert (photo courtesy of ISTH).

devoid of factor X, but containing factors VII and IX, trying various adsorption and precipitation methods, as well as column chromatography. In 1956 we did not have a fraction collector in the laboratory and we spent the nights on a camp bed. An alarm clock sounded every 30 minutes. The research fellow covering the night shift then put a new reagent tube under the column. You can imagine how we were celebrating in early 1958 when we received the first electric fraction collector. After several 80 to 100 hour weeks of intensive (and futile) labour, François smilingly said "do not continue along this line; it won't work. I have done the same experiments for many weeks without results". To my dismayed question why he had not stopped me earlier he calmly replied "You learn by your mistakes, not by your successes".

The existence of a third coagulation factor present in plasma and different from factor VII and IX (factor X) had been postulated by Duckert et al. on the basis of mixing experiment of plasmas from patients with haemophilia B, hepatitis or treated with oral anticoagulants (1). In most of these mixing experiments normalization of the thromboplastin generation test took place that could not be explained by an effect of factor VII or IX (both of these factors could be assayed at that time in our laboratory). Duckert also was able to prepare fractions enriched in factor X. However, it was found – erroneously – that factor X was not stable in plasma. In my opinion, an effect of thrombin in these experiments could not be excluded.

Progressively, the fuzzy factor X story became clearer. In 1955 Telfer, Denson and Wright examined Mrs. Prower, a patient with a mild bleeding disorder (13). The one-stage prothrombin time was in the range of 24 to 30 seconds (a mild defect) and was significantly shortened by the addition of normal plasma, but not by the addition of $BaSO_4$-adsorbed plasma. Therefore the bleeding tendency was initially thought to be due to factor VII deficiency. However, the thromboplastin generation test with the plasma of this patient was abnormal, whereas it was normal in patients with factor VII deficiency. Telfer et al. rightly concluded that the patient's disorder was due to a deficiency of a new coagulation factor. At the same time Cecil Hougie had frequent contacts with Rosemary Biggs and Gwynn MacFarlane in Oxford, where he had learned to perform the thromboplastin generation test. MacFarlane suggested that factor VII deficiency might be a heterogeneous group of patients and mentioned the work of Telfer et al. (11, 13).

In the summer of 1955, Cecil Hougie emigrated to the United States and joined the famous haemostasis research group of Prof. Kenneth Brinkhous in Chapel Hill, North Carolina. He was interested to re-examine patients with factor VII deficiency and decided to use the methods learned in Oxford to restudy Rufus Stuart, a patient previously described by Lewis and Ferguson as suffering from congenital "hypoproconvertinaemia". Rufus's one stage prothrombin time as well as the stypven time were greatly prolonged, and the thromboplastin generation test with the plasma of the patient was abnormal (14). At that time it had been established that the latter two tests are normal in patients with factor VII deficiency.

Conclusive evidence that the defects in the factor VII-deficient patient reported by Alexander and of that in Rufus Stuart were different, was obtained by mixing experiments of the two respective plasmas (15). The prolonged one-stage prothrombin time of each single plasma became normal in the mixtures. Hougie concluded that the newly discovered "Stuart factor" was different from Duckert's factor X, because the Stuart factor was stable in plasma. He felt furthermore that it was also different from the Prower factor, because Telfer et al. had reported that the stypven time was normal in their patient (13). Hougie published his findings in late 1956 (14). More extensive studies done in collaboration with John Graham and Emily Barrow appeared in 1957 (16, 17).

In February 1956 our laboratory in Zurich received citrated blood of a three-week-old child, Delia B., who presented heavy vaginal bleeding and melaena from the 3rd to 7th day after birth. A one-stage prothrombin time was over 60 seconds, and "factor VII" was 2.5% of normal. Blood transfusion and the administration of vitamin K at a provincial hospital resulted in only temporary relief, and the child was transferred to the University Children's Hospital in Zurich. In the following weeks and months many episodes of melaena and cerebral haemorrhage took place, and gradually the child developed a huge hydrocephalus and became blind. Delia died from brain damage and continuous dramatic bleeding episodes before she reached the age of two years.

Coagulation studies in our laboratory revealed an abnormal prothrombin and stypven time, a massively prolonged plasma recalcification time, abnormal prothrombin consumption test, and a highly pathological thromboplastin generation test with the plasma of Delia B. At the end of 1956, we became aware of the publication of Telfer et al. (13) and of the first preliminary report on the Stuart factor by Hougie (14). John Graham sent us lyophilized plasma of Rufus Stuart that permitted us to determine that the Stuart and the Delia plasma behaved identically in all

tests and that no correction of the coagulation defect occurred in mixing experiment. We therefore published our first report in spring 1957 as "Stuart factor deficiency" (19).

I was fortunate to be able to publish our initial studies in the very first issue of the journal *Thrombosis et Diathesis Haemorragica*, the precursor of the present journal *Thrombosis and Haemostasis*. Fritz Koller, Erwin Deutsch from Vienna and Rudolf Jürgens from Basel had just created the journal, and submitted manuscripts were quickly evaluated. In the following months we obtained lyophilized plasma from Mrs. Prower through the courtesy of Dr. Denson. We could clearly establish, by mixing experiments, that the Prower factor was identical to the Stuart and the Delia factor (20). In later experiments it was also found that the stypven time of Prower plasma was prolonged, and it was never clarified why this test gave normal results when first performed.

Fritz Koller then suggested to me to embark on a very large genetic study of the ancestry of Delia B. He felt that a carefully conducted study would be acceptable as a MD thesis at the University of Zurich.

To measure the concentration of factor X in the plasma of family members it was necessary to develop a method that only measured factor X. The Seitz-filtered bovine plasma used for the determination of the "factor VII" measures in fact the logarithmic mean of the factor VII and the factor X concentration (21). I made use of the observation that the one-stage prothrombin time defect of factor VII-deficient plasma is completely corrected to normal values if stypven and cephalin are used in this test instead of brain thromboplastin. I worked out the optimal conditions of cephalin and stypven concentrations, of the method of Seitz filtration, the stability of reagents, and the effect of activation of the "factor X complex" determination by glass surfaces (22). Apparently many researchers were quite happy to use this method since it has been cited over 460 times (23).

The family of Delia B. lives in a small village in the canton Ticino, the Italian part of Switzerland. To get there was about a four-hour drive from Zurich. We installed a small field laboratory in the kitchen of the mayor's house, mainly for centrifuging the oxalated blood and portioning of small amounts of each plasma into several (glass) tubes. These were then rapidly frozen in a container filled with dry ice.

In Switzerland many villages have preserved records on births, marriages and deaths of citizens for hundreds of years. For our patient Delia it soon became obvious that her factor X deficiency originated from a consanguineous marriage. The founder of the factor X deficiency appears to have been born in 1643. We obtained good genealogical information in church and civil records for eight generations (24). The great-great-great-great-grandfather, born 1771, of the mother of Delia was the brother of the great-great-great-great-grandmother, born 1760, of the father of Delia. The pedigree comprised 508 persons. Of these, 75 were identified as living in the United States in 1958. It is probable that even more relatives of Delia were residents in the USA, whose ancestors had emigrated around 1900 because of famine in the valley of the Ticino river. From 150 Swiss and 40 American relatives anamnestic information could be obtained, and from 61 individuals blood samples were obtained. In four persons, exhibiting factor X values between 40 and 70% and presenting a history of poor food intake, blood was redrawn four days after the administration of 10 mg of vitamin K. Factor X levels normalized in two, but remained low around 50% in two others. In 11 relatives factor X levels were below the normal range established in 30 apparently healthy volunteers. All of these were 1st to 3rd degree relatives of Delia: one brother, the father and the mother, uncles and aunts on the father's and mother's side. These individuals were considered to be heterozygous for factor X. Forty-nine samples exhibited normal factor X levels (24).

Factor X was to become a coagulation factor of crucial importance because it occupies a central position in the coagulation cascade. Its activated form in plasma, factor Xa, is inhibited by small doses of heparin. The latter observation provided the basis for the successful and widely used low-dose heparin prophylaxis in patients at risk of developing deep vein thrombosis.

After having completed my training in internal medicine I participated in clinical studies of patients with deep venous thrombosis and/or pulmonary embolism treated with streptokinase. I stayed with my first love in medicine, the study of haemostasis. Like many Swiss physicians I emigrated to the United States in 1961, where I joined the laboratory of Drs. Sherry, Fletcher and Alkjaersig.

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