

Anniversary Issue Contribution

Thrombosis and haemostasis research:
Stimulating, hard work and fun

Margareta Blombäck

Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

To do research is to be inquisitive, to be prepared to set out in unexpected directions in response to new findings – be they negative or positive. Research is like an incurable infectious disease – once you've got it, you've got it for life. A negative result or a result that contradicts what you previously believed often leads to new ideas and inventions. Research also gives you friends all over the world, both collaborators and competitors. Below I mention just some colleagues with whom we performed the basic and clinical research, but rest assured: I have not forgotten anyone.

1950s: Fibrinogen, factor VIII, von Willebrand factor (VWF)

After a few years studying literature and social work, respectively, Birger Blombäck and I, then 24 years old, started studying medicine at Karolinska Institutet, where we are still doing research. In 1951 Birger Blombäck was asked to join the research group of Erik Jorpes, the "heparin man". As Birger's wife I was also allowed to join the group (1). Jorpes had a commission from Vitrum (a private firm later incorporated with Kabi/Pharmacia/Pfizer) to test heparin activity and the analysis required purified fibrinogen. This was prepared from bovine blood, which we fetched from the slaughter house, travelling by tram and later by motorbike, lugging the blood around in large glass bottles. The fibrinogen (prepared according to the methods then in use) was, however, stable only for a few hours. Birger, who has always read the literature more carefully than I, was intrigued by Edward Cohn's idea of plasma fractionation and demonstration of fibrinogen in fraction I. We found that when fraction I was extracted in the presence of high concentrations of glycine, the solubility of fibrinogen was reduced but most of the impurities went into solution. The stable fibrinogen fraction thus obtained we called fraction I-0 (2, 3). The clinical studies described in the following would probably not have been allowed today, but there were no ethical committees at that time.

The first attempt at clinical use of fraction I-0 prepared from human plasma was made on a patient with acquired fibrinogen deficiency; however, the patient went into a very severe fibrinolytic state. We later understood that pyrogens can give rise to fibrinolysis (we had, for the preparation, used water which was not pyrogen free). We knew, however, that Cohn's fraction I contained an antihaemophilic component, so working with Inga Marie Nilsson from Malmö, we tested fraction I-0 for the presence of factor VIII (FVIII). The first sample tested gave a very high yield. Later it was found out that we had used haemophilia B plasma in testing the activity of this sample. We had not yet characterized Stockholm's haemophiliacs in terms of haemophilia A and B. However, it did indeed have 100% yield of the FVIII activity of the original plasma when tested properly.

Dr. Ikuo Yamashina (3), watching me and Inga Marie sitting on either side of a waterbath tilting tubes to study clot formation, said "Maybe or maybe not". I hope he meant this as a joke. We continued to purify fibrinogen and FVIII from fraction I-0 (2–8). Birger also continued with structural analyses of fibrinogen (3).

At the time we were fortunate enough to have good financial support from Erik Jorpes, who had very high royalty incomes from Vitrum (donated tax-free to research). He also received big grants from the National Institute of Health (NIH). Generous support was also provided by Jorpes' friends, the thorax surgeon Clarence Crafoord, and the head of Stockholm Blood Centre, Erik Sköld, who provided the human blood free of charge. Crafoord needed fraction I-0 for those patients who were bleeding due to fibrinogen deficiency (fibrinolysis due to pyrogens?) (3, 4). In order to test the heparin activity in the blood, we participated in the second heart-lung operation ever performed in Stockholm, in 1955.

In 1956 Inga Marie Nilsson had a patient, Birgitta, with severe pseudohaemophilia (in 1957 identified as being von Willebrand's disease [VWD] type 3) who no longer tolerated blood transfusions and whose menstrual bleeding was life-threatening. We decided to try to stop the bleeding with fraction I-0 (containing FVIII). It had to be prepared in a rush. The trains and aeroplanes enlisted to

Correspondence to:
Margareta Blombäck
Dept. of Molecular Medicine and Surgery
Karolinska Institutet
Clin. Chem. Build. L205
Karolinska University Hospital
Stockholm, 17177 Sweden
Tel.: +46 8 5177 4437, Fax: +46 8 312 438
E-mail: margareta.blomback@ki.se

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transport the freshly prepared fraction I-0 from Stockholm had to wait until we got it to them. But it did have a dramatic effect: bleeding stopped, the FVIII level increased and the bleeding time was normalized (4, 8–12). In different studies we tried to figure out why the bleeding time was corrected. Birger and our team prepared the fractions, and Inga Marie was the one mainly in charge of treating the patients. Purified fibrinogen and purified FVIII had no normalizing effect on the bleeding time, whereas fraction I-0 devoid of FVIII or prepared from haemophilia A plasma corrected it. We also observed that the half-lives of FVIII were quite different in patients with haemophilia A versus those with VWD (4, 8) (Fig. 1). In this way we showed the existence of a new “anti-bleeding” factor absent in VWD (by us first called the bleeding time factor) and we named it “von Willebrand factor” (4, 12).

Most of the fraction I-0 that was prepared was given to patients with haemophilia A or VWD. For the first ten years, it was prepared aseptically (as we lost too much of the FVIII in filters used at that time), by the devoted staff in a laboratory where during the first years insulin and peptides were also prepared from animal intestines (3, 4).

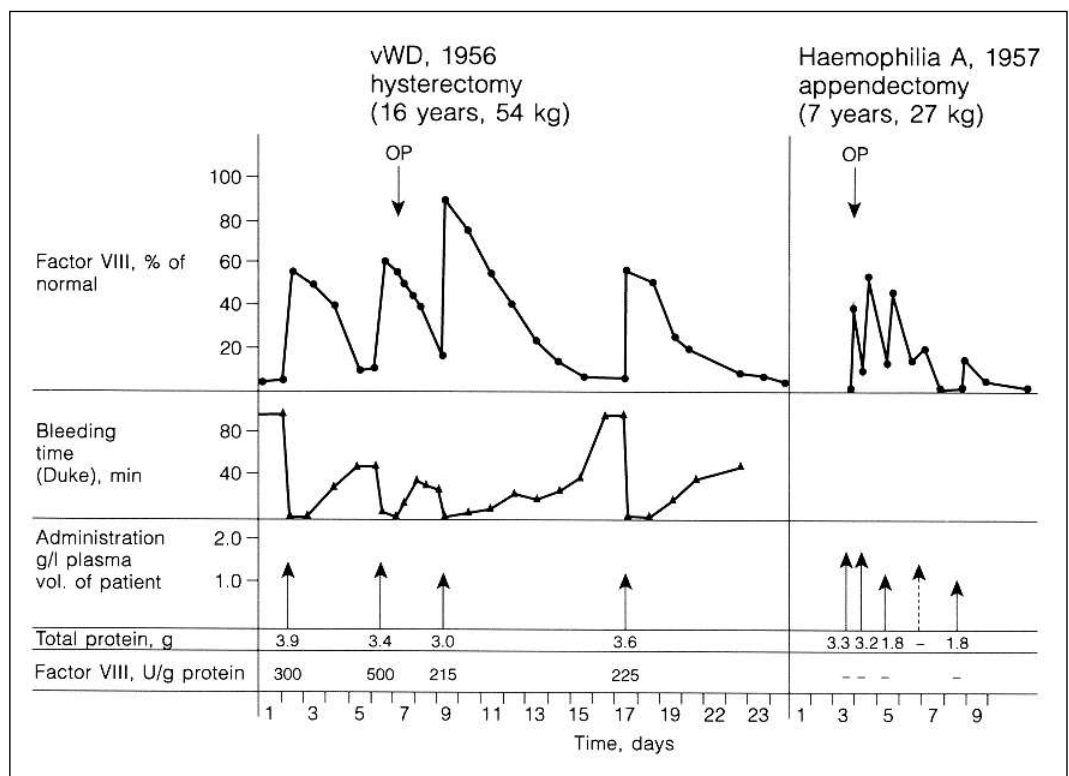
In 1956–1957 Inga Marie Nilsson and I, accompanied by a geneticist, travelled around central Sweden in a car that gave us headaches. (Halfway through the trip we discovered the exhaust system was broken.) Our mission was to study different families with pseudohaemophilia, as we called it then. We visited high and low, rich and poor alike (10). At that time heredity could only be analysed by blood group determination; nonetheless, in two out of ten families we found a (healthy) child whose purported father was not the real father.

The clinical features, heredity and some laboratory findings in our patients were similar to those of patients from the islands

of Åland, who had a form of pseudohaemophilia, described in 1926 by the Finnish doctor Erik von Willebrand (13). Most of the population living in the archipelago of Åland, situated very near Stockholm, are of Swedish origin and speak Swedish. Prompted by Erik Jorpes, who was born and raised there, we took a trip to these islands, to study the surviving members of the original von Willebrand family (for results see below). Because of differences in voltage, the large centrifuge we had brought from Stockholm to spin down platelets did not work, forcing a young doctor to make daily flights back and forth to Stockholm. Every day he flew out with patient blood samples (and duty-free liquor) and worked with Birger to prepare the platelets for the assays (4, 11). The investigation showed that the findings in the original family were the same as those in the patients in Sweden. Before travelling to Åland, Erik Jorpes had met Prof. Rudolf Jürgens on the street and revealed our plans to him, so Jürgens, who was on his way to the islands to hunt seals, of course also brought a team to investigate the same individuals. They found a decreased level of FVIII, just as we did. We could confirm our suspicion that the Swedish patients had the same disease and the same heredity as those from the Åland archipelago (11, 14).

In 1958 Inga Marie, Birger and I were going to present our results at the VIIth International Society of Hematology Congress in Rome. We travelled in our car, which had a lot of problems with its brakes. En route, we passed through Vienna, where we visited another meeting. Later we stayed in a small place on the Italian east coast, happily drinking the Italian wine there and in other small places. Arriving in Rome one day late, we found that Inga Marie's boss (Jan Waldenström) and Erik Jorpes had given us up for lost and were discussing who should present our findings. The congress participants were invited to meet the Pope

Figure 1: Difference in survival time for factor VIII in a patient with severe vWD type 3 (left) and a patient with moderate haemophilia (right) in connection with hysterectomy and appendectomy, respectively. The administered factor VIII is expressed as U/g protein. At the first occasion, thus $3.9 \times 300 \text{ U} = 1,170 \text{ U}$ of factor VIII were given. The broken arrow represents fresh blood administration. (printed with permission from Haemostasis 1992; 22: 239.)



Pius XII, who told us that he was not against abortions if the child was diseased (a haemophiliac).

All through this time we had to earn a living, in addition to studying medicine. I had a job teaching medical biochemistry to medical students. It was not easy as I had almost no basic knowledge except for training in the laboratory. Furthermore, we had to write our theses. Birger's thesis was on fibrinogen. Mine was on antihaemophilic factor (later named FVIII) but Jorpes did not allow me to call it that way. I had to call it "antihaemophilic globulin". We passed our doctorate exams in the spring of 1958, Birger one day earlier than me (5, 8). The day after that, Birger had to give his lecture to qualify as an associate professor. These tight time schedules were orders from Erik Jorpes.

The 1950s were a hard time, but in many respects a very rewarding period. We had a lot of fun and learned to work in teams with both laboratory staff and clinicians.

1960s and early 1970s: Treatment of patients, fibrinogen structure, fibrinogen Detroit, thrombin inhibitors and chromogenic substrates

Of course we had the idea that we could also purify a fraction for treatment of haemophilia B from Cohn fraction II+III. We did so but had to wait a year until we could try it in a patient. Birger, who did the injection, reported that the haemostatic effect was good, but the blood pressure went down to low levels so he interrupted the injection. When newly prepared, the fraction had been tested for adverse effects in a rabbit without problems. Now when we tried it in a dog it showed the same negative effect as in the patient. We suspected that kallikrein activity had developed and was to blame for the adverse effect. Two medical companies have had the same experience when storing similarly produced material. When we wanted to start the production again we could not find our protocols.

While still engaged in production of fraction I-0 for our Swedish patients, we designed a new fractionation plant (see [15]) which was inaugurated by King Gustaf Adolf V and Queen Louise (4). In the beginning of the 1960s I worked mainly with clinical aspects of haemophilia (16) and on carriers of haemophilia in collaboration with Inga Marie Nilsson, Olof Ramgren and others (17). One thing I am very proud of is that we started prophylactic treatment as early as 1958 in a two-year-old boy who is alive to this day (18), though he has had a liver transplant due to hepatitis C. Birger continued to work on the structure of fibrinogen, especially in collaboration with Torvard Laurent, Birgit Hessel and Sadaki Iwanaga (3).

From 1961 to 1962 we spent a year in Australia at the invitation of Dr. Percival (Val) Bailey of the Serum Laboratories in Melbourne to start production of fraction I-0 there and of Dr. Pehr Edman to do protein research (19). When we arrived, Dr. Bailey was no longer in charge. Struggling with an overly restricted budget, he had publicly criticized the government and had therefore been fired (an English tradition). We were shocked. In spite of this we had a wonderful time in Australia and made many friends. We travelled around the continent, lecturing, enjoying ourselves and learning about Aborigine culture. We cherish many memories from that time. Fraction I-0 was used for long time to treat the haemophiliacs in Australia.

In Pehr Edman's laboratory we studied the amino acid sequence of the human fibrinopeptides A (FPA) and B (FPB). When we had returned to Sweden Birger and his team including Russ Doolittle continued to sequence the corresponding peptides from many species to study the evolutionary pattern. I remember lying on the floor at home trying to figure out (by puzzling) the evolution patterns of cow, sheep, reindeer, mink, dog, seal and even elephant from the sequences found. There were no computers or computer programs available for this job at that time. All FPAs included an extremely well preserved sequence in the C-terminal part of the peptides close to the bond split by thrombin. A comparison of the line of reindeer with those of sheep and goat indicated that the latter branched from a deer line rather than from the main bovine line in classical phylogeny (3, 20) (Fig. 2). Birger was invited by Robert G. Macfarlane to lecture about our findings in the Zoological Society of London. Also in the beginning of the 1960s we started to work with Per Olsson on antithrombin and its effect together with heparin. Birger continued to work on the structure of the N-terminal disulphide knot of fibrinogen together with his colleagues mentioned above.

In 1966, with more and more patients seeking diagnosis and advice, I moved from the Karolinska Institute to the hospital (across the road), though I was still an assistant professor connected with the Institute. The production of fraction I-0 was taken over by Kabi, giving us a nice royalty.

In May 1967 we arranged a fantastic meeting on disseminated intravascular coagulation (DIC) in Grisslehamn, a small village on the coast north of Stockholm. Many scientists from all over the world attended the conference. They were a little shocked by the obligatory sauna, but accepted this and other wild Swedish habits, including fermented Baltic herring. After the meeting some of the participants, very special friends of ours, visited an island belonging to Birger and me. Bernadette Verstraete, a guest from Belgium, slipped into the water and Marc-Verstraete went in to rescue her. They were both saved by Per Olsson.

When Dr. Erwin Deutsch, then the editor of *Thrombosis and Haemostasis*, heard about the meeting he suggested that we should publish the presentations of the meeting in this prestigious journal. But when we were going to submit the manuscripts, we learned that a cleaner had thrown the entire – very heavy – box with all the material into the trash (Birger and I were then in New York but our collaborators tried to find them in the Stockholm dump). Later we managed to find all manuscripts but then it was too late to publish them.

In 1967 we spent half a year at the New York Blood Center. While there, we were contacted by Dr. Eberhard Mammen in Detroit, who had a patient, a black girl, with severe menstrual bleedings, who he thought must have an abnormal fibrinogen: Her fibrinogen level was normal, but no clot was formed when thrombin was added (3). As the release of FPA and FPB seemed to be normal we guessed that the amino acid sequence following the FPA sequence was perturbed. We made a tryptic digestion of the A α chain of the N terminal disulphide knot (called N-DSK) and subjected the digest to two-dimensional electrophoresis-chromatography on cellulose thin-layer plates (3). As can be deduced from Figure 3, the tripeptide Gly-Pro-Arg – which in nor-

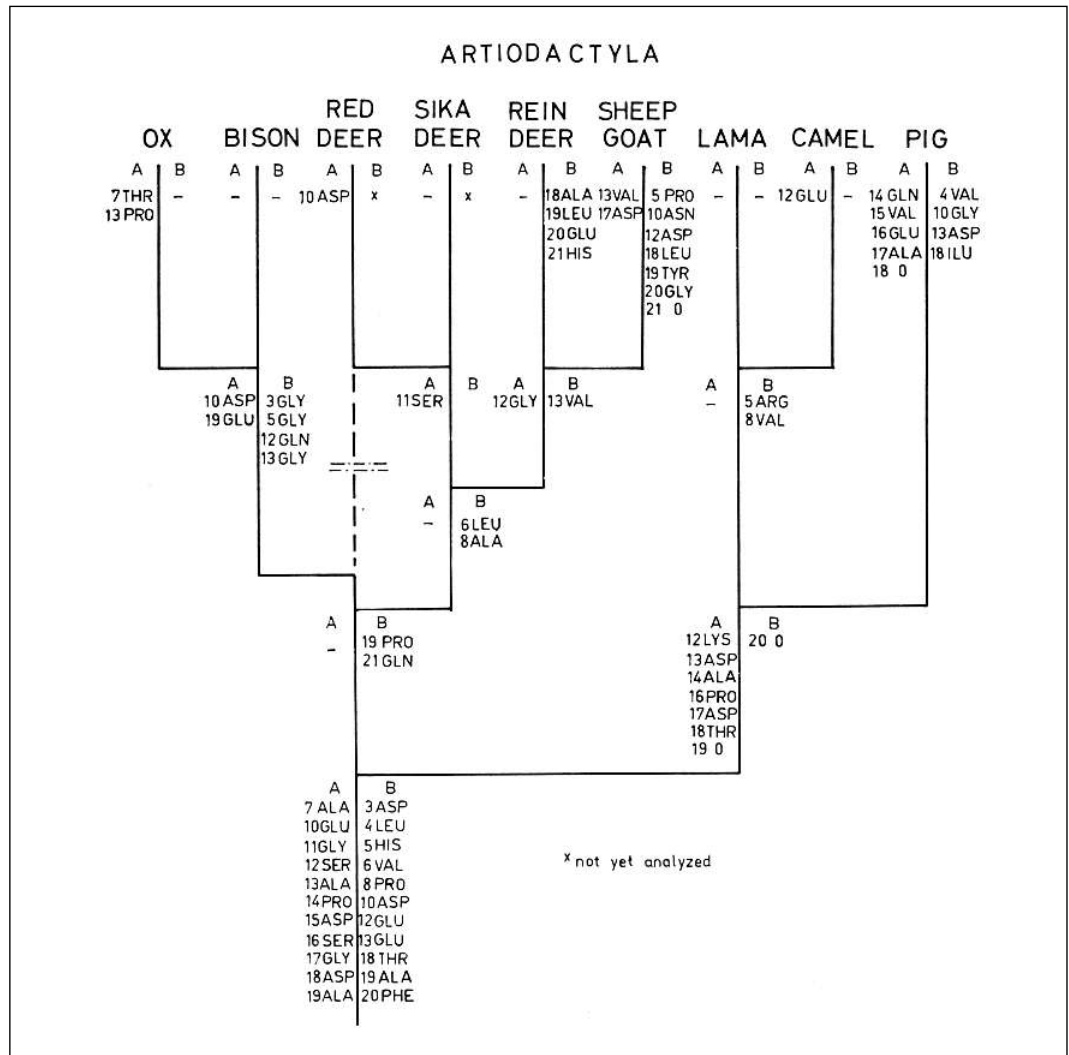


Figure 2: Phylogenetic relationship of different artiodactyls. The relationship was obtained by comparisons of residues at the same position in the different species. (From Arkiv Kemi 1966; 25: 418.)

mal fibrinogen follows the thrombin-susceptible bond of the A α chain after FPA – had moved. Subsequently we could show that the arginine in position 19 of the A α chain had been substituted by a serine residue. We wrote a paper presenting our findings at a lounge of the airport in Detroit (3, 21). On the way to Detroit we stopped at Dr. Oscar Ratnoff's laboratory in Cleveland to give some lectures. We also presented data on the abnormal fibrinogen (fibrinogen Detroit) with the second mutation found in a protein that could be coupled to a defective function (the first was a mutation found for sickle cell haemoglobin, a finding presented in the Vienna meeting mentioned above). Oscar Ratnoff kindly took a colour photo of the gel (Fig. 3).

I loved working in Australia and New York, as in both these places I had the time to work at the bench with my own hands for which I have had almost no time since then.

Early on, we and Inga Marie Nilsson were asked by Bofors Company (Karlskoga, Värmland) to investigate experimentally the function of the snake venom from *Bothrops Jarararaca* (Reptilase) suggested to be used for treatment of bleedings. We then also met some organic chemists there, Göran Claesson and others. However, as part of the C-terminus of FPA seemed to be vital for the thrombin action (see above), we discussed with Göran Claesson and Lars Svendsen (then in Mölndal near Gothenburg – the

firm was later taken over by Kabi) the possibility of using this knowledge for making thrombin inhibitors. They synthesized both the full nona-peptide sequence and shorter variants. The maximum inhibitory effect of these peptides on thrombin was found with the Phe-Val-Arg variant (3, 22). In 1967 the team applied for a patent on the prototype tripeptide for use as inhibitor of thrombin (23). At that time the pharmaceutical industry was not much interested in thrombin inhibitors, though Göran Claesson continued to work partly with the inhibitors when he had moved to Stockholm and later to the UK.

At one of our meetings with Bofors-Kabi in Mölndal the question was put forward "Couldn't we use the tripeptide for measuring thrombin activity?" This was achieved by coupling a chromogenic group, paranitroanilide, to the carboxylic part of the arginine residue in the tripeptide. When the enzyme thrombin acted on this substrate, paranitroaniline was released and the activity (colour) could be measured in a spectrophotometer (24–26). Now we could also measure antithrombin activity. In similar ways, using knowledge of the cleavage sites for factor Xa, plasmin, trypsin, kallikrein, and other proteases chromogenic peptide substrates were constructed and in a few years

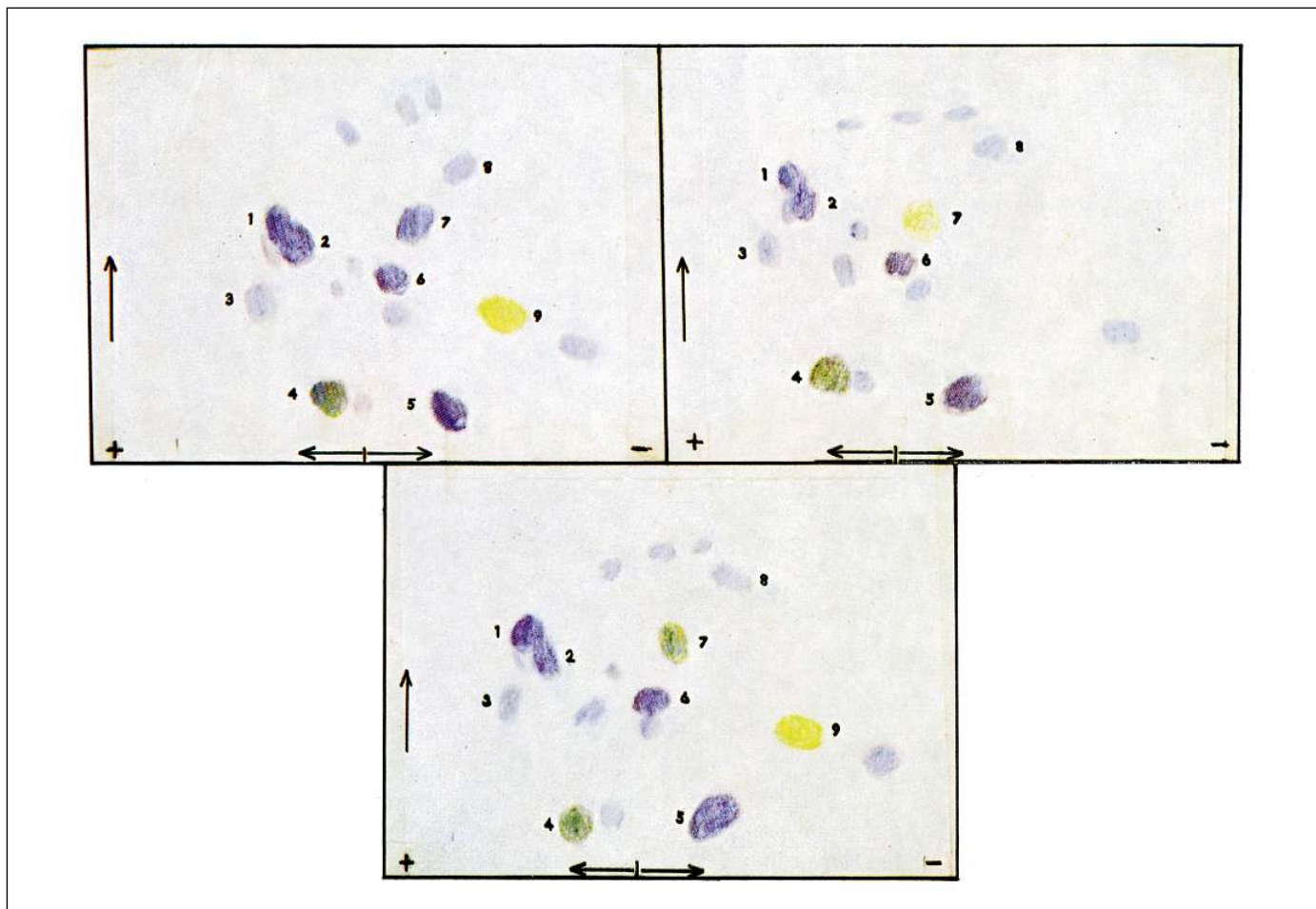


Figure 3: Two-dimensional electrophoresis (\longleftrightarrow)-chromatography ($\downarrow\uparrow$) of tryptic digests of α -chain fragment. Top left: normal fibrinogen. Top right: fibrinogen Detroit (homozygote). Bottom: fibrinogen Detroit (heterozygote). Photos taken by Oscar Ratnoff. Part of normal $\text{A}\alpha$ chain:.....PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-**ARG-GLY**-PRO-**ARG**-VAL VALThrombin splits between **ARG-GLY** when releasing FPA. In fibrinogen Detroit **SER** substituted **ARG**. (From NRF Hématologie 1970; 5: 671-678).

there appeared more than 1,000 papers where different substrates for measurement of proenzymes, enzymes and their inhibitors had been used.

Looking back, I cannot understand how we could get so much done during the 1960s and early 1970s. We owe a lot to the excellent collaborators (3, 4) mentioned above and to Agnes Henschen and our foreign visitors Ikuo Yamashina, Sadaki Iwanaga, Jane and Rick McDonagh and Gene Murano, Barbara Kowalska Loth to name a few. While Birger continued the studies on fibrinogen and related topics, I had to devote most of my time to the clinical side because of my position at the hospital. I got many young collaborators from different clinics (6) who produced doctoral theses together with Birger and me or me alone as supervisor.

Late 1970s, 1980s and 1990s: Clinical work, teaching, purified VWF, HIV, hepatitis C

After our sojourn in New York, Birger spent much time as a visiting professor at the Blood Center, New York. We continued to

work together after our divorce in 1972, though until I retired in 1992, the clinical work as head of a coagulation laboratory took most of my time.

My laboratory diagnosed haemophilia, VWD, and other bleeding disorders, but also did investigations in thrombophilic patients. We were on call to handle all such patients, including patients bleeding in the intensive care (DIC etc). We did laboratory analyses and medical controls of haemophilic patients from a large part of Sweden and took care of them all when they had to be hospitalized for operations or similar problems. We took special care of the haemophilic children in Stockholm as well. Together with Barbro Wiechel, who was in charge of the out-patient care of adults, we introduced prophylactic treatment as well as self-treatment for adult patients with severe and moderate haemophilia and with VWD type 3 in 1976. For the patients the possibility of self-treatment gave a sense of great freedom.

My lab was one of the first in Europe to start using instruments for clotting factor analyses; the laboratory assistants told me I did this just to save staff. I also introduced a good computerized system very early for the hospital accountants, but when my laboratory was fused with Clinical Chemistry, the head of the de-

partment said we had to go back to do it by hand again. I did a fair amount of teaching especially trying to regularly upgrade a small book called “Coagulation News” on clinical diagnosis, laboratory analyses and treatment of haemostatic disorders.

I had a highly dedicated staff and many good medical collaborators. I especially want to mention Nils Egberg from my laboratory, Hans Johnsson from the medical clinic, Dag Nyman (see below), Margareta Hellgren and later Katarina Bremme from the gynaecology clinic, Julius Soreff from the orthopaedic clinic, and Sixten Bredbacka from the intensive care unit, but there were many others, among them several thoracic surgeons (6). In the beginning I had a rather high royalty income from Kabi for our inventions and I could therefore support the research work in the laboratory. However, afterwards I regretted I had too little time to support the collaborators intellectually and I wasted much money unnecessarily, using it for routine work.

But my patients have also been very good collaborators. I had one very devoted patient with severe haemophilia A, whom I could not persuade that he needed treatment with FVIII concentrate: He knew I needed his plasma for analyses of FVIII. Nils Egberg and I were working with the possibility of treating patients with thrombotic disorders with Defibrase or Arvin (Ancrod) but we first wanted to find out if it gave rise to increased bleeding tendency so we tried Defibrase in this patient. The patient reported only a slightly increased capillary bleeding.

HIV and hepatitis C

In the summer of 1982, Frank Schnabel, the head and creator of the “World Federation of Hemophilia”, came to Stockholm for a short visit. He told me, “Meta, there is a terrible disease spread by blood products – I am sure this will be a catastrophe for haemophilic patients.” In 1983 at the XVth World Federation of Hemophilia Congress in Stockholm (27) there were several lectures on AIDS and the immunological status of haemophiliacs, though there were not yet any tests for HIV. In Sweden we had been forced to use FVIII products from the USA in order to treat our patients, as the supply of fraction I-0 was insufficient (the plasma was from Sweden and Finland). Also the patients preferred the American product as it could be injected in a small volume. Sadly, 100 of 136 patients with severe haemophilia A got infected with HIV and about 70% died, of those many young boys. When we started treating haemophiliacs with fraction I-0 the mean age was 16 years, but before the HIV-era appeared the mean age was near that of the healthy population. In 1979 the Swedish Board for Technical Innovation had started a program to try to provide more Swedish plasma, but the resulting increase in plasma volume for fractionation did not reach the patients until HIV was a fact. The HIV infection was clearly a disaster for the patients but was also extremely hard for the mothers who had injected the FVIII concentrates into their children. Our VWD patients did not have HIV as they were still treated exclusively with fraction I-0 produced from blood from Sweden and Finland where HIV infection at that time was low.

Already in the early days we had noted that administration of fraction I-0 could give rise to hepatitis but as the therapy saved lives I think we did not bother so much about this infection. In later years we became aware what hepatitis C could do to our patients, and now it was not only haemophiliacs but also those with

VWD whose health was at stake. Some of them have died of liver damage before good treatment was available.

Von Willebrand disease again

In the beginning of the 1980s, Birger and Thorell designed a good method for preparation of VWF (28). This preparation showed by sequence analyses, for the first time, that VWF was a single chain protein (29). We tried it on two patients in whom it indeed had an extraordinarily beneficial effect (30). We offered the procedure to Kabi but they answered too late. The Finish Red Cross then started to prepare the fraction. However, due to the procedures for inactivating HIV it did not retain activity. Norbert Heimburger at the Behringwerke in Marburg (Germany) early devised a pasteurization method for FVIII and other concentrates in order to get rid of hepatitis virus (31). This resulted in that most of the haemophilic patients in Germany could be treated with a product free of HIV.

Way back when I was writing my thesis I was certain that patients with VWD type 3 were homozygous, though I was unable to persuade either my opponent or Inga Marie Nilsson that I was right. However, I had a secretary, Elisabeth Söderlind, who had kept track of all VWD families, and, guided by this material, geneticist Maria Anvret and I started investigating the polymorphisms using DNA linkage analysis. We found that patients were homozygous in five families, and were either compound heterozygous or bore a new mutation in three families (32). After much discussion with an editor, who earlier had rejected our papers on polymorphisms in haemophilia A and B, this paper was published in another journal. I often wonder if editors prolong the review processes because they want to publish their own results first. In 1991, Zhiping Zhang a young Chinese, came to our departments in Karolinska Institutet. He had stated that he had read all my papers on VWD (later he confessed he had only read a recent abstract).

My triumph

In 1992 at the XXth World Federation of Hemophilia Congress in Athens, Ian Peake said in his lecture that it was impossible to find any mutation in the VWF gene. In the discussion I presented part of what we had found in Stockholm (Fig. 4). The same year, Zhiping Zhang published two important papers (33, 34), and his findings were summarized in his thesis (35). By using PCR technique followed by direct sequencing he found a single cytosine deletion in exon 18, nonsense mutations in exons 28, 32 and 45 and nine other new mutations (33–35) (Fig. 4). The deletion in exon 18 is the most common one found in the Swedish patients. Heredity investigations showed that it can be traced back several hundred years. I believe it originated from immigrants or sailors from the South. The nonsense mutation in exon 28 was found in patients whose parents came from Finland.

In the meantime Dag Nyman, who then was working in my laboratory, had shown that the families from Åland that were suggested to have VWD, could be divided into several categories (36). The survivors of the original family had the characteristics of what I prefer to call VWD type 1, similar to the relatives of the Swedish patients with VWD type 3, i.e. decreased level of VWF and ristocetin cofactor activity in addition to normal and decreased levels of FVIII; the platelet aggregation was normal. As

in the Swedish patients, one cytosine deletion was detected in exon 18 in the surviving relatives. We investigated a hair from a small boy with VWD type 3. He was homozygous for the deletion (37).

At present I would like to study how the mutants especially those in exon 18 and in exon 28 have travelled in Europe, but this is too much work for an old lady and the money is not available.

Fibrinogen, fibrinogen structure again, arteriosclerosis, influence of acetyl salicylic acid (ASA) and global assay of coagulation

By studying the fibrin gel network in 1981 and 1989, Birger showed what influenced the final fibrin network structure in a pure fibrinogen system (3) and also later in a plasma environment; he stated that the clot structure is influenced by the first formed network (38). This could of course be of interest for arteriosclerosis research, and, indeed, Anders Hamsten and I, examining this phenomenon, found that the initial network was much tighter in males who had experienced myocardial infarction at a young age (39). Since then much of my research work has been in this direction, especially together with the groups of Håkan Wallen and Gun Jörneskog and my postdoc Shu He. In this respect the influence of acetyl salicylic acid (ASA) has also attracted my attention. I found that low doses of ASA make the network more porous and that it can thus be degraded more easily by plasmin (40). I think this finding is of much greater importance than platelet researchers admit.

For many years I have been frustrated by the belief – apparently shared by many – that problems in the complex haemostasis system can be detected by measuring a single factor or inhibitor. Although I am very proud of my work together with Anders Hamsten and Björn Wiman on plasminogen activator-1, I have had a great interest in developing a global haemostasis assay. Even though many global assays are now entering the market, I am still working on this subject together with Shu He and others.

Thrombin inhibitors again – what we are doing at present

After the first trials of thrombin inhibitors (see above) Astra-Zeneca, together with one of Birger Blombäck's former PhD students Ann-Catrine Teger Nilsson, continued the work and devel-

oped a very promising oral thrombin inhibitor (41) that could be used for short-term prophylactic treatment against thromboembolism. However, the administered drug led to liver damage in a few cases and was withdrawn. During the past few years new thrombin and factor Xa inhibitors have been developed and launched onto the market. The hope is that they will be good enough to replace both heparinoids and coumarin derivatives.

My team is presently exerting much effort to investigate some inhibitors with our new methods (the fibrin network method and the global assay), hoping the results will help the clinicians to find out about both the favourable and the unfavourable effects of these new treatment methods (42).

Other areas we worked in, missed opportunities, SSC work

In retrospect I see I was often interested in too many aspects. On one occasion Birger and I purified coagulation factor V, probably due to that we considered it an important factor in haemostasis. Birger and I were always interested in antithrombin and its mechanism and we did much interesting work with our great friend Per I. Olsson who has devoted the last two decades to work on heparin-coated surfaces for clinical application. A very good collaborator of mine in the antithrombin area is also my good friend Ulrich Abildgaard, whom I forgot to include in a paper on that subject. A paper by Petter Friberger on heparin cofactor II was sent to the chief editor of Thrombosis Research – Birger himself – who probably thought it was just for comments from him personally, as there was no answer until after reminding him, so we missed the opportunity of being one of the first writing about this topic in the literature.

A grave scientific mistake-information is difficult even to those around you.

I have made many studies on the influence of hormones on haemostasis. In a study on the menstrual cycle I found a great increase in VWF and FVIII in the luteal phase, especially in one volunteer being my laboratory assistant for many years. Though my conclusion in the paper was correct that female hormones can influence the level of these coagulation factors it was exaggerated. Almost 10 years later I found out my laboratory assistant had been running to the place of blood sampling, allowing to draw alternative conclusions about the relation between exercise and haemostatic status.

For many years I have been interested in what happens during storage of plasma for transfusion purposes or for fractionation of factor concentrates. I enjoyed the work with Joanna Chmielewska on this topic and recently Anna-Maija Suontaka from my group published a thesis on the subject (43). I think the issue has been looked into far too little by blood transfusion specialists.

For several years around the start of the 1990s I co-chaired and chaired the Scientific and Standardization Subcommittee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH). I enjoyed the work, though it was not easy to get 20 subcommittees to agree on new nomenclature issues (published in 1994 but still not used by all in full). Without the dedication of the Executive Secretary Cathy Cole and the support from the Executive Director Harold Roberts of ISTH, the work would not

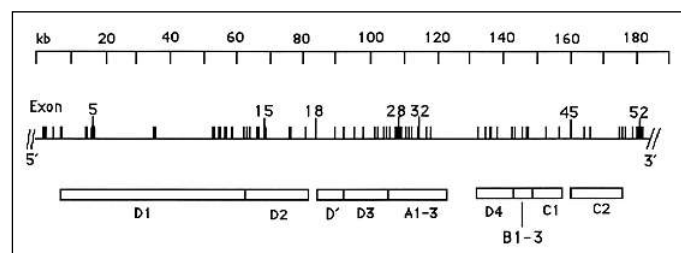


Figure 4: Structure of the VWF gene and corresponding protein domains. Mutations and polymorphisms found by Zhiping Zhang are indicated. For details see references 33–35, 37. (Printed with permission from Zhiping Zhang.)

have been possible. In this regard an early great mentor for me and a friend was Rosemary Biggs, a former editor-in-chief of *Thrombosis and Haemostasis*.

Concluding remarks

The area of haemostasis has widened tremendously since the 1950s. It has given me much pleasure to be allowed to take part both in the basic and the clinical work. I always liked to work

with and stimulate young doctors to do research. To be able to apply basic research findings in the clinic, and to be able to solve questions from the clinicians, is a prerequisite for further development of the area, and will be of benefit for the patients.

Acknowledgement

Birger Blombäck has kindly read the manuscript and corrected it in places where he thought I was wrong.

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