43rd Annual Scientific and Standardization Committee Meeting

June 6-7, 1997

Florence, Italy
## Table of Contents

- Registry of Animal Models of Thrombotic and Hemorrhagic Disorders ........................................ 2
- Biorheology ........................................................................................................................................ 4
- Contact Activation............................................................................................................................ 7
- Control of Anticoagulation .................................................................................................................. 9
- Exogenous Hemostatic Factors Subcommittee: Registry ................................................................. 15
- Factor VIII and Factor IX .................................................................................................................. 18
- Factor XIII ......................................................................................................................................... 22
- Fibrinogen and DIC ............................................................................................................................ 25
- Fibrinolysis .......................................................................................................................................... 28
- Hemostasis and Malignancy ............................................................................................................. 31
- Lupus Anticoagulant/Phospholipid-Dependent Antibodies ............................................................ 34
- Perinatal/Pediatric Hemostasis .......................................................................................................... 37
- Plasma Coagulation Inhibitors .......................................................................................................... 39
- Platelet Immunology .......................................................................................................................... 43
- Platelet Physiology ............................................................................................................................. 47
- Predictive Variables and Cardiovascular Disease ............................................................................... 49
- von Willebrand Factor ....................................................................................................................... 51
- European Committee for Standardization/Comité Européen de Normalisation (CEN) ............... 54
Registry of Animal Models of Thrombotic and Hemorrhagic Disorders

Chair: L. Badimon, Spain
Co-Chairs: T. Griggs, USA; G. Johnson, USA; D. Ginsburg, USA;
        L. Drouet, France

J.J. Badimon reviewed the present status of models to study local delivery devices. It is suggested that pig carotid artery is a necessary step before the application to the pig coronary to study local delivery.

L. Drouet presented four models in four different animal species to study thrombosis and surface passivation. It is suggested that a fiber of fibrin/fibrinogen plays a major role in passivation.

K. Mohlke (for D. Ginsburg) presented mouse genetic resources: genome project tools and transgenic mice. The indications and the cautions for the use of these genetically manipulated mice was discussed. References to Internet access to the mouse genome and transgenic animal databanks will be included on the Animal Model Subcommittee home page on the Internet (www.med.unc.edu/isth/).

G. Johnson presented the document of recommendation on "Restenosis: Which Animal Model is the Best?" by L. Badimon, T. Griggs, and himself on behalf of the Subcommittee. The discussion helped to solve some additional questions such as the following:

- Is there a best large animal model?
- What is the optimal sequence of pre-clinical studies?
- How close are we to a small animal model of atherosclerosis?

After an extensive discussion, the chairman proposed that the name of this subcommittee be changed to Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis. This was approved.

The next meeting will be held in Ljubljana, Slovenia, with future subjects:

1. Gene transfer and regulation of gene expression in animal models
2. Review of models of venous thrombosis and pulmonary embolism which could give rise to a document of recommendation for models of venous thrombosis and pulmonary embolism.
The biorheology subcommittee met to present (I.) three working party reports, and (II.) three new topics for our new working parties, as well as to consider (III.) future directions. Part I reports will be reviewed by our committee for submission to the SSC for publication in *Thrombosis and Haemostasis* in the coming three months. Part II reports will be prepared for final presentation at the next SSC meeting in Ljubljana in June 1998. Future directions are listed below. Attendance was excellent with up to 100 participants.

I. **Working Party Final Reports**

Dr. Jeff Hubbel reported on a theoretical and practical analysis of flow chambers used in biorheological studies of thrombus formation. Model calculations for parallel-plate chambers were calculated for platelet surface deposition at shear rates of 100, 500, and 1500/sec, with emphasis on physical effects of red blood cells for platelet diffusion to surfaces. Theoretical and real data were compared for platelet-collagen deposition. Again, red blood cells dominated the "axial dependence" for platelet deposition on parallel-plate surfaces; the macroembolization process was also analyzed.

Dr. Steve Hanson reported on the dependence of antithrombotic drug efficacy on flow. He provided an excellent review of antithrombotics targeted at clotting as well as anti-platelet receptors and demonstrated the critical observations showing the dependence of the inhibitory effects of these drugs on the shear rate operating at surface-cell-cell interactions. In particular, von Willebrand factor was shown to be increasingly important with increasing shear rate, with the extent of platelet activation and secretion being important determinants. Effects of different receptor partners were also considered. Controlled blood flow conditions simulating thrombogenic physiologic environments may be particularly useful for understanding drug mechanisms and for predicting drug efficacy.

Dr. Gordon Lowe presented exhaustive statistical analyses on risk factor parameters for thrombotic vascular events, based on the Edinburgh Artery study (five-year follow-up) of 1500 patients. Plasma viscosity was shown to be an independent predictor for vascular disease, independent of plasma fibrinogen concentration, hematocrit and LP(a). The mechanism(s) underlying the predictive behavior of blood and plasma viscosity elevations remain a scientific challenge.

II. **Topics for New Working Parties**

Drs. Y. Ikeda and Y. Cadroy reported on effects of flow on endothelial cell functions: coagulant and fibrinolytic activity (Y.I.), and antithrombotic activity (Y.C.). Dr. Ikeda reviewed use of HUVEC’s in a modified cone-plate viscometer to show gene
upregulation in thrombomodulin by shear stresses of 18 d/cm² for 20 hrs exposure. Differential effects of shear on changes in tissue factor and tPA by shear, and modulation by cytokines (IL1 and TNF) were presented. Dr. Y. Cadroy introduced the use of parallel perfusion chambers with coverslips coated with ligands like P-selectin or HUVEC, and direct \textit{ex vivo} blood perfusion led to thrombi measured with distance from perfusion cell entry point. This distance determined the state of "blood activation." Thrombomodulin activity was varied in different ways and correlated with fibrin and thrombus formation evaluated at 50/sec shear rates.

Drs. Hubbel and Hanson reported on local drug delivery and flow. Dr. Hubell reviewed the theoretical and real observations on use of implanted materials on blood vessels to provide highly permeable "depots" for drugs with a low permeability barrier (cap) on the luminal side. Dr. Hanson predicted and gave preliminary experimental results for using helical catheters (stent-like) to "stream" drugs on the surface of blood vessels. Drugs can thus be targeted at the wall re: thrombosis, with up to 100-1000 times less drug needed than conventionally used via total blood access protocols. Similar results were presented for vascular graft releasing drugs from an integrated drug delivery system.

Issues relating to studies of blood cell adhesion and aggregation in flow were finally reviewed by five speakers, including shear and platelet testing at bedside.

Dr. Frojmovic emphasized that the state of activation of platelets used (both preparation and activator-dependent) determined the shear rates needed to support shear-induced aggregation. The state of platelet activation also determined the actual ligands and platelet receptors dominating in these adhesive interactions driving shear-dependent platelet aggregation. Thus, von Willebrand factor secreted onto platelet surfaces mediates aggregation without any externally added ligand for ADP-activated platelets at shear rates from 300-1500/sec, in contrast to shear rates of 60000-10000/sec needed for "resting" platelet shear-mediated aggregation driven by soluble von Willebrand factor. Differential roles for the platelet receptors GPIIb and GPIb/IIIa were also described depending on the increasing shear rates. Dr. Ikeda reviewed "resting" platelets sheared in plasma which he has studied in cone-plate viscometers, identifying the roles of soluble fibrinogen and vWF, and the platelet receptors (GPIb and GPIIb/IIIa). Issues of better definition of platelet activation in shear and effects of shear exposure times were recognized.

Dr. de Groot demonstrated the critical importance of comparing platelet adhesion to proteins immobilized on surfaces for static versus shear-dependent effects. VWF was unique in supporting platelet deposition continuously with shear rates upwards of 2000/sec. Roles of GPIb and vWF were analyzed, including adhesion of platelets onto different collagens. The use of platelet detachment experiments with increasing shear stresses for analyzing firmness of adhesion onto different protein surfaces was described.

Dr. Gerard Nash reviewed the flow system used for following neutrophil transient and firm attachment to platelets immobilized on flat glass capillaries expressing P-selectin. Issues of cell preparation quality, shear rate windows analyzed, and nature of surfaces
used were carefully addressed. Low platelet surface coverage was seen to provide efficient neutrophil deposition, with rolling and neutrophil activation as prerequisites for firm capture.

Dr. D. Varon described a new flow device, the cone plate-let analyzer (CPA), which allows quantitation of platelet deposition onto microtiter wells coated with ECM at shear rate of typically 1500/sec. Fresh citrated whole blood (as little as 150 microliter) is added, "stirred" with a cone added to the well for 2 mins, and surface coverage and particle size are then analyzed with image-analysis computer interface (all potentially at bedside). Examples were given for monitoring von Willebrand disease patients for diagnosis and therapy, as well as for monitoring anti-thrombotic drug efficacy in patients with cardiovascular disease.

Standardization issues were discussed for all the above presentations, including a need to compare fresh blood and different anticoagulants.

III. Future Directions

1. It was agreed that a new working party would be formed on "rheological bedside devices for monitoring and managing vascular diseases: current status and future needs," with Dr. Frojmovic initiating this.

2. A need for a party on "standards in rheological studies of cell adhesion" was also recognized, though this will be integrated mostly in all future reports.

3. An interest in a working party on "studies of von Willebrand factor in cell adhesion in flow" was also strongly expressed.
Contact Activation

Chair: A. Schmaier, USA
Co-Chairs: H. Saito, Japan; B. Lämmle, Switzerland; P. Walsh, USA;
W. Müller-Esterl, Germany

I. Standardization of Contact Factor Assays:

The subcommittee has focused activities on standardization of contact factor assays. Contact has been made with Trevor Barrowcliff, Elaine Gray, and Tony Hubbard to obtain the SSC plasma for standardization. Initial efforts have been made toward this process.

Plasma factor XII enzymatic and antigen assays were performed by Drs. Jones, Gallimore, and Winter of the United Kingdom. The SSC plasma was determined to contain about 1 U/ml. Drs. Schousboe and Rojkjaer of Copenhagen, Denmark, performed similar studies. There was excellent correlation between their work and the group from the United Kingdom.

Dr. Cheryl Scott of the United States presented her efforts in determining plasma kininogen levels. Using a fluorescent antigen assay, she is able to determine plasma levels of total and high and low molecular mass kininogen. The SSC plasma contains about 60 μg/ml high molecular weight kininogen (HK). Dr. Schmaier's laboratory using coagulant and antigen assays also determined the SSC plasma to contain 1.02 U/ml and 58 μg/ml plasma HK in the SSC plasma.

Dr. de la Cadena of the United States used an amidolytic assay to measure plasma prekallikrein in the SSC plasma. He determined it to contain 0.92 U/ml prekallikrein in plasma. Dr. Schmaier's lab, using a different assay, determined similar values, 0.92 U/ml PK activity and 33 μg/ml PK antigen in the SSC plasma.

It was agreed among the committee that a working group be formed to begin a standardization process of contact proteins (factors XII and XI, prekallikrein, high molecular weight kininogen) of the SSC plasma. A working group (Drs. Jones, Scott, Schmaier, Schousboe) will be formed, chaired by Dr. Schmaier, to develop guidelines for plasma collection for activity and antigen assays for contact factors and to determine levels of each of the contact factors by activity (IU/ml) and antigen (μg/ml) by each of the independent forms of assays in investigators' laboratories. It is planned that each investigator will use his own pool of NHP for the standard and test the SSC plasma against this standard.

II. International Patient Registries:

Efforts are underway to determine both retrospectively and prospectively the natural history of patients with contact protein deficiencies. National registries from Italy and Sweden were examined. Dr. Berrettini of Italy reviewed the Italian registry for evidence
of contact protein deficiencies. Thirty-seven patients with homozygous deficiency (factor XII levels < 5 percent, evidence of heterozygous defects in both parents) were identified. Eighteen of these patients have a history of thrombosis; six of these patients had bleeding histories. Dr. Bylesjo of Sweden indicated that there is a Swedish registry and stated that he has begun to obtain access to this information for analysis of contact protein deficiencies.

A working party of Drs. Schmaier, Berrettini, Bylesjo, and Castleman was created to provide guidelines for collection of data and criteria for the definition of homozygous or heterozygous deficiency of a contact protein.

III. New Assays of Contact Proteins:

A session presented by Dr. Hasan of the United States was devoted to development of new contact protein assays based upon the developing biology and function of contact proteins. Using HK as an example, Dr. Hasan indicated how platelet aggregation, calcium mobilization studies, flow cytometry, peptide cleavage studies, and solid phase binding assays can be utilized to measure HK's thrombin inhibitory function. It was emphasized that as new biologic activities of contact proteins are appreciated, new assays to measure these proteins can be developed.
Control of Anticoagulation

Chair: T. W. Barrowcliffe, UK
Co-Chairs: G. Agnelli, Italy; A.M.H.P. van den Besselaar, The Netherlands; B. Boneu, France; S.M. Lewis, UK; L. Poller, UK; F.E. Preston, UK; A. Tripodi, Italy

Current Tasks

The chairman welcomed the participants, numbering over 300, and outlined the program which was divided into three sessions and based on the tasks as agreed upon at the last subcommittee meeting:

Heparin

1. Development of reference plasmas
2. Development of recommendations on monitoring of LMW heparin

Thrombin Inhibitors

Review of collaborative study data on Ecarin time

PT Standardization

1. Establishment of new WHO human thromboplastin IRP
2. Work toward recommendations on calibrated plasmas
3. Review of revised WHO requirements on thromboplastins

HEPARIN

I. Unfractionated Heparin

Dr. Gray gave a progress report on the development of reference plasmas with defined activities of unfractionated heparin. The effects of high fibrinogen and FVIII concentrations on APTT and anti-Xa assays were described, since such high levels are often found in patients. It is intended that pilot batches, consisting of pooled patients' plasmas, ex vivo plasmas from normal volunteers, and modified in vitro spiked plasmas, will be prepared in the near future and subjected to an inter-laboratory study during the coming year.

Dr. Gray also announced that the WHO standard for unfractionated heparin would require replacement during the next year, and WHO is organizing a joint meeting together with the United States, European, and Japanese pharmacopoeias to devise suitable materials and methods.

II. LMW Heparin

Dr. Giles described the activities of the working party which had been formed last year to deal with the issue of monitoring LMW heparin. The Working Party consisted of Dr. Giles (Chairman), Drs. Samama, Andrew, Boneu, Hemker, Ofosu, and Nesheim and has
divided its activities into three areas: A) monitoring in children, B) monitoring in adults, and C) methodology.

A. Monitoring in Children

**Dr. Andrew** emphasized that laboratory monitoring of LMW heparin in children should be considered separately from that in adults because of the following:

1. Different pharmacokinetics
2. Greater risk of bleeding with underlying primary disease
3. Possible development of renal compromise and/or acquired coagulopathy
4. Long-term treatment
5. Possible dosage errors with home treatment

Because of these issues, it was considered by the working group that monitoring LMW heparin in children is important both for prophylactic and therapeutic use. The appropriate timing and frequency, and the therapeutic ranges for children have not been determined with certainty, and, as with adult monitoring, the methodology is still a matter for discussion, though in practice the anti-Xa method has mostly been used.

B. Monitoring in Adults

**Dr. Samama** reviewed the clinical relevance of laboratory monitoring of LMW heparin in adults. Platelet monitoring is important for both prophylaxis and treatment because of the risk of thrombocytopenia, which although lower than with unfractionated heparin, is still present.

Measurement of LMW heparin levels in prophylaxis is neither necessary nor useful except in special cases, such as very low or very high body weight, renal insufficiency, or the occurrence of a bleeding or thrombotic episode.

In treatment of established DVT or pulmonary embolism, several published studies found no relationship between anti-Xa levels and clinical outcome, but other studies did find a positive relationship between anti-Xa and either efficacy or bleeding. At present, there is insufficient data for a definite conclusion on the clinical utility of monitoring in these indications and further clinical studies are needed.

Until now, anti-Xa assays have been mostly used, though for practical reasons rather than their demonstrated superiority.

C. Methodology

**Dr. Nesheim** described the heterogeneity of LMW heparins with regard to their molecular weight binding to antithrombin and activities against FIIa and FXa. He
concluded that it was necessary to measure both types of activities to characterize any sample containing LMW heparin.

In discussion, Dr. Hemker agreed with Dr. Nesheim and emphasized the difference between methods to measure LMW heparin concentrations (anti-Xa and anti-IIa) and methods to measure their effect on the coagulation system such as the thrombin potential which was still under development. Dr. Harenberg commented on the utility of the Heptest for both LMW heparin and unfractionated heparin monitoring.

Dr. Giles concluded by indicating that, despite some uncertainties, the Working Group should be able to establish practically useful recommendations in this area which, subject to agreement by the Subcommittee and the SSC, could be published. It was agreed that the working group would prepare written recommendations during the coming year with a view to presentation and final adoption by the Subcommittee at next year's meeting.

**THROMBIN INHIBITORS**

Dr. Rübsamen presented the interim results of a collaborative study on the ecarin clotting time (ECT) for the detection of PEG hirudin. Twelve laboratories participated and eight had returned data. The results were somewhat disappointing in that despite the use of the same ecarin reagent in all laboratories, there was a wide variation in the slopes of the dose response lines, and, in two laboratories, the samples gave very long clotting times or were incoagulable. It was considered that freezing and thawing of the samples may have contributed to their variability and that another study with lyophilized samples might give better results.

Dr. Fareed reviewed the use of the ECT in clinical settings. It was found that the ECT was not affected by concomitant use of oral anticoagulants, or by heparin up to 1 iu/ml. There was a poor correlation with APTT, and this was considered to be due to the more specific nature of the ECT. For the peptide inhibitor Argatroban the HPLC method did not correlate well with the ECT--this was found to be due to a metabolite with reduced but still detectable anticoagulant activity.

Dr. Fareed concluded by emphasizing that, although the ECT was useful for monitoring a variety of thrombin inhibitors, each agent required its own standard.

In discussion, it was agreed that, whilst the ECT is clearly a useful test, further work to reduce inter-laboratory variability is necessary before it can be recommended as the method of choice.

**PT STANDARDIZATION**

**Collaborative Studies:**
**Dr. Tripodi** presented the final report on the establishment of the human recombinant thromboplastin, to be labeled rTF/95, as the new WHO International Reference Preparation for human thromboplastin, to replace BCT/253. Following endorsement by the Subcommittee last year, a report was submitted to WHO, and the material has now been officially established. A manuscript has been prepared by Dr. Tripodi and this has been approved by the Subcommittee as an SSC publication in *Thrombosis and Haemostasis*.

**Dr. Hubbard** gave a final report on the collaborative study on proposed European reference plasmas for INR. As described at the last meeting, there were significant differences in INR results with the three IRPs; the INRs with BCT/253 and RBT/90 were similar, but those for OBT/79 were lower. The majority of participants preferred to combine INRs of BCT/253 and RBT/90, keeping the data on OBT/79 available separately, and this proposal has now been incorporated into a final report which has been sent to the European Commission. It is hoped that the reference plasmas will be officially established by the end of 1997.

**International Perspectives:** Two presentations were given describing the practice and problems of control of oral anticoagulants in different countries.

**Dr. Uhetsuka** (Japan) presented on behalf of Dr. Sakuragawa. Seventy percent of Japanese hospitals use the thrombotest for anticoagulant control. Results are converted into INRs by means of an equation. Good correlation is claimed between INR and thrombotest. The usual therapeutic range is 2.5-3.5. Bleeding tends to occur when the derived INR>3.0. The bleeding risk may be accentuated by frequent concomitant usage of antiplatelet drugs.

**Dr. Kabaeva** (Russia): Russia has its own thromboplastins. These are derived from human brain tissue and have an ISI close to 1.0. Monitoring of oral anticoagulant control is performed by determination of the prothrombin index. Phenindione is the most frequently used oral anticoagulant agent. Warfarin is unknown. Only 1:15,000 people receive OAT. This compares with an estimate figure of 1:200 in Europe and the United States. Russia would welcome assistance from the Subcommittee in this area.

**Dr. van der Meer** reported different methods to determine therapeutic quality control of OAT. He recommended the method developed by colleagues in Leiden. He also indicated that this method could be adopted to define appropriate therapeutic ranges in different patient populations. Dr. van der Meer described the establishment of a new European group whose main activity would relate to clinical aspects of oral anticoagulant control. Those interested should contact Dr. van der Meer.

There was strong support for the chairman's expressed view that future meetings should include more clinical aspects of OAT.

**European Concerted Action on Anticoagulation**
**Dr. Poller** presented results from the field study of European Concerted Action on Anticoagulation. Effects of coagulometers were presented in some detail. The study emphasized the need for an additional step using certified lyophilized plasmas to provide local ISIs.

**Dr. Houghton** presented a comparison of calibration exercises comparing orthogonal regression and linear regression analyses. The results suggested that the simpler linear regression method gave a reasonable approximation to orthogonal regression and was worthy of further study.

**WHO Guidelines** (information in this report's Appendix).

**Dr. van den Besselaar** gave a synopsis of the important changes and additions to the revised WHO Requirements on Thromboplastin, which had been discussed at a recent WHO consultation meeting. The revised document prepared by the drafting group of Dr. van den Besselaar, Dr. Tripodi and Dr. Poller, has now been circulated to the members of the Subcommittee for comments. Any additional comments should be sent in writing to either the Chairman or Dr. Padilla at WHO by the end of June.

**Calibrated Plasmas**

Due to time constraints, there was no time for the final presentations and discussions in this area. The chairman proposed that a working group be formed with the task of drafting recommendations on the preparation and use of calibrated plasmas. This was agreed and it was suggested that this should be a major task for the Subcommittee in the next two years. The working group would consist initially of Drs. Barrowcliffe, Poller, Houbouyan, Taberner, van den Besselaar, and Johnston. Others interested in contributing should get in touch with the chairman.

**ADDITIONAL TOPICS**

Three additional topics had been suggested that could not be incorporated in the current programme. There were i) citrate concentration for PT, ii) review of duration of oral anticoagulant therapy, iii) review of adverse events in oral anticoagulant treatment.

Considering the earlier discussions on the inclusion of more clinically oriented topics, the chairman proposed to ask the SSC if the remit of the Subcommittee could be broadened and possibly the name changed to "Subcommittee on Anticoagulants." A longer time for the next meeting could be requested to allow for additional topics.

**FUTURE TASKS**

The following future tasks were agreed upon:

**Unfractionated Heparin**: Reports on reference plasmas and WHO standard to be presented in 1998.
**LMW Heparin:** Recommendations on monitoring to be drafted by the working group, for prospective approval at 1998 meeting.

**Oral Anticoagulants:**

WHO Requirements-- Comments to WHO by end of June 1997.

Calibrated Plasmas-- Preliminary report by working group at 1998 meeting.

Clinical Aspects-- New tasks on reviews of duration of therapy and adverse events to be initiated.

**Appendix: WHO Guidelines:**

A revision of the WHO guidelines was initiated in April 1997. A draft document was written after a consultation at WHO headquarters in May 1997. Definitions are given for tissue factor, thromboplastin, prothrombin time, prothrombin time system, mean normal prothrombin time (MNPT), prothrombin time ratio, international sensitivity index (ISI) and international normalized ratio (INR). It is recommended that the manual ISI of a thromboplastin should be in the range 0.9-1.7. Four types of PT system calibration are distinguished as follows: 1) calibration of International Reference Preparations, 2) calibration of secondary reference materials, 3) calibration of manufacturer's preparations against the corresponding secondary (in-house) standard, 4) local PT calibration. The principle of like-to-like calibration is maintained. Secondary human reference thromboplastins should be calibrated against rTF/95, secondary rabbit against RBT/90, and secondary bovine or combined thromboplastins against OBT/79. It is recommended that calibration of secondary reference thromboplastins should be carried out by at least two laboratories. Calibrations of type (1) and (2) should be performed with fresh normal and fresh coumarin samples. Calibration type (3) may be carried out with frozen lyophilized plasmas if it is shown to provide the same results as with fresh samples.
Exogenous Hemostatic Factors Subcommittee: Registry

Chair: F. Markland, USA
Co-Chair: N. Marsh, Australia

Seven members of the registry were in attendance plus about 8-15 guests.

Welcome: Francis S. Markland, Chair
N. Marsh, Co-chair

Introduction of new member of the registry: Dr. Carmen Arocha-Pinango

Outline of the program:
- Reports on Inventories
- New Business
- Next Meeting
- Change of Chairmanship

Reports on Inventories

Dr. Pirkle was unable to attend to present his updated inventory of venom thrombin-like enzymes (TLEs), but his remarks were given by Dr. Markland.

There are eight amino acid sequences of thrombin-like enzymes that have been determined and they were presented as a figure. All share active site residues H57, D102, and S195 (the numbering of the sequences for all these enzymes is based on that of chymotrypsinogen). One problem is with ancrod for which two sequences have been reported. One derived from cDNA and the other chemically determined. There is a high degree of difference between the two sequences owing to the fact that the cDNA sequence was determined from a probe directed to the amino-terminal end of the protein, thereby producing potential ambiguities. All 12 half-cystine residues aligned perfectly. Carbohydrate content in TLEs varies widely. Reclassification of A. rhodostoma to C. rhodostoma seems accurate based on recent evidence. A table of all the thrombin-like enzymes and various properties were presented. A motion was made to submit this report to the SSC for publication. The motion was seconded and approved.

Dr. Markland updated the inventory of fibrinogenolytic and fibrinolytic enzymes.

He discussed seven groups based on the snake families from which the enzymes are derived. The grouping is further broken down into geographical areas. Sixty-five enzymes were described. One plasminogen activator was additionally reported. The metalloproteinases, which are mainly Aα-chain preferring, all show structural homology.
An example was shown, using the predicted structure of fibrolase, which has the metzincin motif in the active site. Tables showing the properties and cleavage specificity were presented to the registry. A major change from the past inventory was a reclassification of the enzymes based on snake family and geography rather than on chain cleavage. Additionally, a number of serine proteinases with fibrino(geno)lytic activity directed primarily to the Bβ-chain were reported. One suggestion was to indicate that the enzymes presented are direct-acting agents. A motion was made to submit the report to the SSC for publication and the motion was seconded and approved.

Dr. Brinkhous was not present and has resigned from the registry. Thus, his report on platelet aggregating agents was not presented.

Dr. Teng described factors affecting platelet aggregation.

These are arranged according to activities into four different groups: GPIIb-IIIa antagonists, collagen platelet interactive agents, non-coagulant non-enzymatic inducers of platelet aggregation, and effectors of vWF-GPIb interaction. Additionally, sources of platelet aggregation factors from plants were presented and these were arranged into 12 groups based on activity. Dr. Teng desires not to update his inventory at this time and this was accepted by the chair.

Dr. Arocha-Pinango described hemostatic activities of arthropods.

Dr. Arocha-Pinango stated that the information is very scattered and there are very few well-characterized agents but those that have been identified are divided into five groups. The first are effectors of thrombin-fibrinogen interaction. There are those that act on platelets and in this group there is a single agent which is an inducer of aggregation. Prothrombin activators are not well-characterized. Fibrinolytic agents are mostly identified as plasminogen activators, but no further information is known. Lastly, a large number of enzymes fall into the category of miscellaneous unclassified agents of unknown mode of action. Many of the papers describing these agents were published a number of years ago, and additional information is unavailable. A motion was made to submit Dr. Arocha-Pinango's report for publication to the SSC and this motion was seconded and approved.

New Business

The chair called for recommendations for replacement of Dr. Brinkhous. One recommendation was previously received and another was suggested from the floor. The new member of the registry will be appointed by the new chairman.

Next Meeting

It was proposed that the next meeting of the registry take place in Washington, DC, in 1999, unless any pressing matters arise between now and then. The inventories in need of
Updating at the next meeting are Platelet Aggregation Agents, an inventory of bacterial fibrinolytic agents, and an inventory of snake venom hemorrhagic proteins.

Change of Chairmanship

This meeting concludes Dr. Markland's term of chairmanship. Dr. Marsh thanked Dr. Markland for his service as chairman. The meeting was then adjourned at 5 p.m.
Factor VIII and Factor IX

Chair: I.R. Peake, UK
Co-Chairs: D. DiMichele, USA; J. Ingerslev (Secretary), Denmark; K. Mertens, The Netherlands; C. Prowse, UK; Y. Sultan, France; A. Yoshioka, Japan

The audience was welcomed by the chairman who introduced the meeting and outlined the programme.


Collaborative study on assays of activated FIXa by E. Gray et al., Thrombosis and Haemostasis 1996, 76, 1114-1117.

Final Stages:

Nijmegen modification of the Bethesda assay. A. Giles et al.


Final Report:

The use of porcine FVIII in infants and children - J. Lusher. To be produced after inclusion of further material from the UK.

Reports of On-going Activities:


Viral transmission by blood products in noninfected patients by PCR technology - E. Berntorp, one year to go before final recommendation would be possible.

New Proposals:

Registry of factor VIII/IX concentrates - C. Kasper. No report given. Chair has received a draft. Final form next year.

Protease inhibitors and bleeding in haemophilia - E. Briët.

Case report presented. Participant meeting arranged for p.m. to consider recommendations and reporting.

Recommendations on the use of PUPs and PTPs - G. White.
Proposal forwarded to study PTP instead of PUP in clinical immunogenicity trials. Comments to Dr. White within four weeks. Proposal to be written.

Risk Factor Assessment for Inhibitor Development:

Product-related Risk. Y. Sultan, chairman.

Incidence of FVIII inhibitors in PUPs treated with recombinant FVIII: Introduction - Y. Sultan. Dr. Sultan summarized recent French data on inhibitor occurrence.

Data from France and USA - C. Rothschild. Dr. Rothschild gave a detailed report on a French study of 53 PUPs treated with rVIII. D. DiMichele reported on local US and PR cohort comparing two rVIII treated groups with patients shifted from plasma-derived factor VIII to recombinant.

Proposal for a French-American-International Registry - L. Aledort, Y. Sultan. The questionnaire was outlined by Dr. Aledort. Additions to include genetic information and patients treated with a plasma-derived product. Current and past inhibitors should be requested also. Dr. C. Hay also outlined preliminary data on UK PUPs.

Inhibitors in mild and severe haemophilia - F. Rosendaal. Reviewed Dutch patients estimating the spontaneous occurrence of inhibitors and those related to particular product.

Genetic Risk. L. Hoyer, Chairman

Kogenate inhibitor study - J. Lusher. Outlined proposed study which will commence soon.

Recombinate inhibitor study - A. Goodeve. Detailed the present genetic information on the Recombinate study.

Immunogenetic risk - C. Hay. Outlined situation and stressed that ethnic differences make studies very difficult.

Risk and Assay Variability. D. DiMichelle, Chairman

Inhibitor assay variability in Canada - A. Giles. A positive inhibitor result may be influenced by methodology adopted, type of equipment, and type of deficient plasma. Chemically depleted FVIII deficiency plasma should not be used.

Inhibitor assay variability in UK - S. Kitchen, E. Preston. Dr. Kitchen reported the UK NEQAS study that showed wide variation.

Discussion followed. Nijmegen modification of the Bethesda assay to be endorsed by the SC. Attempts to reduce cost of materials to be explored by Dr. Giles. Working group to meet soon to study in detail the possibility of preparing guidelines and definitions of inhibitors for possible publication to assist in the prediction of inhibitor development. Commercial companies to be asked to contribute.

**Standardization Issues. C. Prowse (UK), K. Mertens (NL), chairmen.**

FIX Standards. Dr. M. Weinstein reported on the finalized work on a new FDA/EP standard for Factor IX concentrate, and Dr. E. Gray reported on the development of a new activated FIX standard (FIXa) (potency not yet established).

FVIII Standards. Dr. M. Weinstein reported on the ongoing work with a new factor VIII concentrate MEGA standard (MEGA II). Multicentric potency estimation to be performed. Dr. T. Barrowcliffe reported that new WHO standards for plasma and concentrate FVIII will be prepared during 1998 for final release during 1999.

Discussion followed. Members expressed hope that harmonization between US and WHO standards could be reached.

**Collaborative Studies: results of SSC studies.**

**Report of 1996/1997 studies. S. Raut reported on two FVIII studies (SSC3 & SSC4) of FVIII at two different potency levels. Recombinant & intermediate purity concentrate show a wide inter-laboratory variation. Factor VIII concentrate study to be continued for another 12 months.**

**Plasma and Concentrate Units and in vivo Recovery.**

Summary of the Problem. T. Barrowcliffe. The problem was outlined. Post-infusion Factor VIII:C levels are subject to variation dependent of the method adopted to measure FVIII (one-stage or chromogenic substrate method).

A Fractionater's View. M. Mikaelsson. Data presented indicating that concentrate added to haemophilia plasma (e.g., after injection) does not compare well with a plasma standard but compares more accurately with a concentrate standard.

A Clinician's View. C. Lee. Data presented (UK Hemofil M & Recombinate pharmakokinetic study) illustrated that potency assessment of a concentrate as well as the quantitation of VIII:C in post-dosage samples gave higher values with chromogenic substrate methods than one-stage procedures.

Discussion. Dr. J. Lusher stressed the importance of assay discrepancies as demonstrable in pharmacokinetic analysis, showing data signifying an increased peak FVIII:C value as well as an apparently prolonged in vivo half-life value when the chromogenic assay was
used. Dr. Lusher further suggested a clinical review of databases of PUP and PTP recombinant studies to search for a bleeding/dosage relationship.

**Recommendations. Dr. T. Barrowcliffe argued that the reasons for assay discrepancies are not understood and proposed to the SC**

1. that recovery should be based on one-stage assays until more information is obtained;
2. that post-infusion samples should be assayed against the concentrate standard, preferably the same material as was infused; and
3. that the concentrate standard should be diluted in hemophilic plasma (preferably from the patient).

**In conclusion, it was hoped that further studies would help to overcome these problems. The possibility of dosage/effect studies will be explored.**
Factor XIII

Chair: L. Muszbek, Hungary
Co-Chairs: P. Board, Australia; C. Greenberg, USA; A. Ichinose, Japan; J. McDonagh, USA

Attendance: about 100 attendees.

1. Nomenclature

At an earlier meeting of the Subcommittee a proposal for the recommended terminology concerning blood coagulation factor XIII had been presented and discussed. At that time, the Subcommittee suggested slight modifications and asked L. Muszbek (Hungary) to resubmit the modified version to the Subcommittee for approval. The revised version was presented by L. Muszbek and discussed by the members of the Subcommittee. The proposal consists of three parts, the main points are outlined below:

a. Nomenclature of blood coagulation factor XIII (FXIII) from different sources.

Blood coagulation factor XIII present in the plasma (having the tetrameric structure A_2B_2):

plasma FXIII, (pFXIII)

Blood coagulation factor XIII present in cells (in platelets, megakaryocytes, monocytes and macrophages and having the dimeric structure A_2):

cellular FXIII (cFXIII)

Recombinant cellular factor XIII:

recombinant FXIII (rFXIII)

b. Designation of blood coagulation factor XIII subunits.

Recommended term for the potentially active subunit present both in plasma and cellular FXIII:

A (FXIII-A)

Recommended term for the inhibitory subunit present in plasma but not in cellular FXIII and also present as non-complexed free form in the plasma:

B (FXIII-B)
c. Designation of activation intermediates and end-products of blood coagulation factor XIII.

Active form of blood coagulation factor XIII in general: FXIIIa
Thrombin-cleaved inactive form of plasma FXIII: pFXIIIa'
Thrombin-cleaved inactive form of cellular FXIII: cFXIIIa'
Thrombin-cleaved active form of FXIII: FXIIIa*
Non-cleaved active form of FXIII: FXIIIa¡
Thrombin-cleaved inactive form of subunit A: A'
Thrombin-cleaved active form of subunit A: A*
Non-cleaved active form of subunit A: A¡
Activation peptide cleaved off from the A subunit: AP-FXIII

Points (a) and (b) were unanimously approved by Subcommittee members, point (c) was supported by the majority. Regarding point (c), some questioned the need for such a detailed proposal on activation intermediates while the supporters argued that abbreviations for activation intermediates and end-products are already in use in the literature and the lack of accepted terminology causes considerable confusion. L. Muszbek was given the task of preparing the final form of the manuscript which will then be submitted to SSC for vote and publication.

2. Registry of FXIII deficient patients

A European registry has been set up by the European Thrombosis Research Organization Factor XIII Working Party. The question was whether to expand the European registry to make it a world-wide registry and thus make it a Subcommittee activity.

R. Seitz (Germany) gave an account of the present stage of the registry and outlined certain points of clinical appearance and supplementation therapy of FXIII deficiency based on the information provided by the survey. H. Mikkola (Finland) presented the results of a comprehensive molecular genetic investigation of FXIII deficient patients that was made possible by the European registry. A. Ichinose (Japan) and P. Board (Australia) provided additional information on FXIII-deficient patients in Japan and Australia, respectively.

Although a world-wide registry on FXIII-deficient patients did not seem a realistic goal for the near future, the creation of a database on the molecular genetic defects of FXIII-deficient patients was approved by the Subcommittee. A. Inbal (Israel) volunteered to set up the database.

3. Further issues

R. Ádány (Hungary) reviewed data on the site of synthesis of FXIII subunits and made methodological recommendations for further studies on this area. P. Bishop (USA) presented an animal model of inflammatory bowel diseases which seems to be applicable for studying the beneficial effect of FXIII concentrate in such cases. C. Greenberg (USA)
provided data on the use of site-directed mutagenesis in studying structural-functional aspects of FXIII subunits.
Fibrinogen and DIC

Fibrinogen Chair: M.W. Mosesson, USA
Co-Chairs: F. Brosstad, Norway; M. Matsuda; W. Nieuwenhuizen, The Netherlands

DIC Chair: F. Taylor, USA
Co-Chairs: M. Blombäck, Sweden; M. Kazama, Japan; T. Matsuda, Japan; I. Bokarew, Russia; J.W. Ten Cate, The Netherlands; N. Sakuragawa, Japan

Dr. M. Mosesson chaired the first portion of the meeting dealing with fibrin sealants as chair of the fibrinogen subcommittee. Drs. M. Blombäck and F. Taylor chaired the second portion of the meeting dealing with soluble fibrin assays as chairs of the DIC subcommittee. The subjects reported and discussed by this subcommittee were as follows:

I. Fibrinogen Subcommittee

There was a wide ranging discussion concerning the characterization and rationale for standardization of fibrin sealants including present and past formulations, delivery devices, clinical applications. Discussants included Dr. D.L. Amrani (session moderator), Dr. U. Martinowitz, Dr. T. Seelich, Dr. M. Weinstein, Dr. M. MacPhee, Dr. M. Nowartarski. Approximately 120 persons were in attendance.

There is currently a broad range of formulations for fibrin sealants, which vary greatly with respect to the concentration of fibrinogen, factor XIII concentration, fibronectin concentration, and the presence and amounts of a number of other plasma constituents and/or additives. Specific issues that were raised included 1) the need for high fibrinogen concentrations; 2) the need for standard assay of mechanical strength and standardization of in vitro methods for measuring fibrin sealants; 3) the role of factor XIII; 4) the inclusion of antifibrinolytics and other agents. There is no accepted or preferred basis for such formulation, or objective criteria by which preparations are measured.

The discussants agreed by consensus that it would be valuable to develop a rationale and establish criteria for standardization and calibration of fibrin sealants. A working party was formed which will exchange written communications among interested participants and develop criteria for characterizing fibrin sealants as outlined above. Dr. Michael Mosennon will head up the working party. It is anticipated that information will be solicited and collected over the next six months, disseminated for consideration, and be ready for discussion at the next meeting of the SSC in 1998.

Dr. F. Dati (Behring Diagnostics) reported on a study of criteria for establishing a High Concentration Fibrinogen Standard. Dr. Dati presented a detailed analysis of the need for a high fibrinogen standard, problems in the measurement of fibrinogen, and standardization of testing methods. He made specific recommendations for a high fibrinogen plasma standard. It is expected that Dr. Dati will submit a written summary of his report for further consideration by the Fibrinogen Subcommittee.
II. DIC Subcommittee
   A. Immediate, Practical Issues
      1. Definition of DIC

         In 1994-95, Drs. Müller Berghaus and Margareta Blombäck oversaw the development of a definition of DIC by the subcommittee: DIFF (DIC), Disseminated Intravascular Fibrin Formation, is an acquired process associated with disseminated soluble fibrin formation within the microvasculature.

      2. Assay of Soluble Fibrin Formation

         In 1996-97, Dr. Charles Francis enlisted the assistance of the DIC subcommittee and of Drs. F. Taylor and M. Blombäck in the development of a protocol for the assessment of the clinical relevance of assays of soluble fibrin using four different commercially available ELISA assay kits. A protocol was agreed upon which included collection of samples from patients with DIC (i.e., patients with culture positive sepsis or with specified trauma within 12 hours of injury) as well as from patients following myocardial infarction (Thrombo Study). During the first year (1996-97) of this two-year study, 12 members of the DIC subcommittee agreed to participate. Four members provided samples from 17 sepsis patients and Dr. Owenings provided samples from the 44 trauma patients. The results are summarized as follows:

         PERCENT OF RESULTS WHICH WERE ELEVATED (SOLUBLE FIBRIN)

<table>
<thead>
<tr>
<th>SOLUBLE FIBRIN ASSAY #</th>
<th>THROMBO (275-892)</th>
<th>TRAUMA (44)</th>
<th>SEPSIS (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>95</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>70</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>73</td>
<td>81</td>
</tr>
</tbody>
</table>

         The biochemical/immunologic rationale explaining the differences between these four tests, although of importance, is secondary to simply collecting data on the overall incidence of elevated concentrations of soluble fibrin in these three arbitrarily defined clinical conditions. It was agreed with Dr. Francis that at least 20 additional sepsis DIC samples be provided by Drs. Blombäck and Bredbecka (Sweden), Bokarew (Russia), and Sakuragawa and Wada (Japan) as well as possible additional samples from Drs. Levi (Netherlands), Brenner (Israel) and Falanga (Italy).
3. **Assessment of Markers of Pre DIC**

The remainder of the meeting was taken up by presentations by Dr. Hoots concerning his experience with anti-thrombin concentrates in neurotrauma patients and by Dr. Wada concerning his experience with assays of the pre DIC state in patients who went on to develop DIC (114 leukemia, 126 non-leukemic patients). Dr. Wada observed that hemostatic markers such as PAP (PIC), TAT, D-dimer and soluble fibrin that were already elevated began a further significant rise two to three days before the onset of DIC. On the other hand, protein C, protein S, and tissue factor levels did not change. It was agreed that continued examinations of markers of increased but compensated hemostatic activity (pre DIC) might be as important as evaluation of a definitive marker of DIC or decompensated hemostatic activity such as soluble fibrin. In line with this, Dr. Ten Cate and Dr. Taylor suggested that a controlled study using primates (baboons) also be considered as a means of evaluating assays of soluble fibrin and enzyme/inhibitor complexes as markers of decompensated and compensated responses of the hemostatic system, respectively. Accordingly, Drs. Taylor, Wada, and Sakuragawa agreed to run assays for soluble fibrin and for enzyme/inhibitor complexes including PAP (PIC), TAT, APC/PCI, TFPI/Xa, and for Factor VIIa on blood of baboons infused with very low (10^2 CFU/kg), low (10^5 CFU/kg), medium (10^-7 CFU/kg), and high (10^10 CFU/kg) concentrations of E. coli. The question is whether the above markers are of value in discriminating between a stressed but compensated hemostatic system (pre DIC) and a stressed and decompensated system. In theory, enzyme/inhibitor complexes consisting of regulators and mediator components might appear under conditions where the hemostatic system is stressed but compensated before markers of DIC such as soluble fibrin, DD dimers, or FDP appear. These studies will be done this year.

B. **Less Immediate, Conceptual Issues**

Assuming these studies described above are informative, Dr. Bokarew raised the serious question of whether we can provide a picture or concept which could be used 1) to interpret these studies, and 2) to connect the various clinical conditions in which we will find evidence of DIC (soluble fibrin) with the molecular and cellular events unique to each condition which can be understood and used by the practicing physician. It was agreed among the chairs of the subcommittee to draft a preliminary report on this issue by the 1998 ISTH-SSC meeting in Slovenia. jwd/6/10/97
Fibrinolysis

Chair: C. Kluft, The Netherlands

Attendance fluctuated between 80-120 persons.

**Blood collection and handling procedures** (Moderator: Dr. I. Walker)

Dr. I. Walker summarized the items that were rather well established and focused on (a) residual items that should be considered and were not well documented, (b) a future report aiming at defining minimal requirements, taking into account haemostasis assays as broad as possible (fibrinolysis, coagulation, platelets). She questioned whether the SSC should take over the recommendation of ICSH to use uniformly one citrate concentration being 0.109 mol/l. Dr. J Conard introduced the issue of influence of menstrual cycle. Dr. B. Polack summarized critical items of quality control of the increasingly used CTAD tubes and identified the absence of external quality control on such specialized tubes. Mr. P. Meijer showed data that the low pH in Stabilyte tubes might interfere with the assay system by lowering the pH. Dr. M. Stegnar identified the nearly complete absence of data on stability of analytes upon storage of plasma and showed some preliminary data suggesting the importance of this item.

It was decided that Dr. Walker should proceed to prepare a proposal for minimal requirements, as above, which should be circulated among other subcommittees to provide a broad basis for discussion of the proposal next year. In addition, a report form accompanying blood collection will be drafted for discussion (Drs. Walker and Kluft).

In addition, subgroups will work on filling in gaps in knowledge that are considered of importance. Dr. Conard with the assistance of presently known volunteers, Drs. Stegnar, Kluft, Jespersen, van de Ende and Douglas, will produce a report on fluctuations of haemostasis factors during the menstrual cycle. A practical approach of recording the time of the last menses will be included in the aforementioned report form.

Dr. Stegnar, with the assistance of Drs. Gaffney, Douglas and Kluft, will further evaluate how studies on stability of stored samples should be organized. Dr. Kluft will submit a request to selected scientists, both within and outside the fibrinolysis field, to ask for data they might have on stability of stored samples. This matter was considered urgent in view of the use of the SSC secondary standard, and Dr. Jespersen suggested that specific attention be paid in this respect to lyophilization and other potential matrix problems.

**SSC Secondary Standard: Report on assignment of a value for t-PA and PAI-1 antigen** (reported by Drs. Gram and de Maat)

Using the NIBSC standard as a calibrator for the methods, values were assigned to the SSC secondary standard. Five companies with commercially available kits reacted very positively and provided free reagents. Unfortunately, the results between the methods used disagreed strongly by a factor of three to five for t-PA antigen and by a factor of two for PAI-1 antigen. No value could therefore be assigned. It was decided that the assistance of the company scientists would
be requested to find the reason for the discrepancies and a renewed discussion about establishment of a reference method might be needed.

The assignment for plasminogen and antiplasmin was delayed to await the introduction of a new NIBSC batch of the required standards and is expected to be available next year. Dr. Gaffney reported to have ampouled a new plasmin standard (97/536) to succeed 77/588 and also a new glu plasminogen standard to replace the British standard 78/646. It was decided to seek WHO approval for the glu-plasminogen standard, as well.

**Assignment of a value to the t-PA antigen plasma standard** (reported by Dr. Gaffney)

The subcommittee agreed with the proposal of the assigned value on the new plasma t-PA antigen standard (94/730). Dr. Gaffney will propose this to the WHO. It was further agreed that information on the variation in detected t-PA antigen in the assignment study and information about the added amount of t-PA should be accessible for users.

**Assignment of an antigen value to the PAI-1 standard for activity** (reported by Dr. Gaffney)

The activity standard 92/564 established by the WHO showed in a collaborative study a variation in results too wide to be acceptable. The subcommittee agreed with Dr. Gaffney's suggestion not to proceed to WHO.

**Update on the scu-PA standards and methods of assay** (reported by Dr. Gaffney)

New batches of non-glycosilated (NG)(95/564) and glycosilated (G) (95/668) standards for scu-PA show excellent stability at six months (20¼ and 37¼ C). Measurement (after activation) directly on chromogenic substrate shows virtually identical contents of both preparations in agreement with amino-acid analysis. Expression in SI units (katal) is now possible. It was decided that this was now the preferred method for comparison with other preparations. The previous discrepancy with tests of *in vitro* biological activity was strongly reduced by introduction of a clot lysis assay using exogenous lysis instead of endogenous lysis. The warning was issued not to confuse *in vitro* resemblance and standardization of NG and G with thrombolytic efficacy which might depend on other factors as well.

**Criteria for specificity of methods and methods of testing: Plasmin inhibitor and t-PA antigen**

Mr. Meijer assisted by Drs. Hanns, Christensen, Wiman and Kluft had prepared a report and a set of criteria for specificity and proposed methods of testing. Dr. Booth, assisted by Drs. Chandler, Ersdal, Rijken, Kinnby and Stegnar, did so for t-PA antigen. The subcommittee added suggestions and finally approved the criteria and methods for both analytes. Both groups will prepare a full report for publication, which will be reviewed by the PGM before further processing within SSC.

The working group on Scu-PA (Drs. Binder, Dooijewaard, Gunzler, Benraad) issued an interim report and will report next year.
In view of the assignment of the SSC secondary standard, it was decided to form a new working group for plasminogen which will report next year (chairman and participants to be invited).

**Nomenclature of genetic polymorphisms**

Dr. M. de Maat, on behalf of a working group (plus Drs. F. Green and N. Sala) on genetic methodology formed within the frame-work of ETRO (Population genetics of hemostatic risk factors for arterial vascular disease), summarized the nomenclature that should be used for intron and exon mutations in genes of interest for vascular diseases when the recommendations of Beaudet and Tsue (*Human Mutation* 1993; 2: 245-8) were followed. It was felt it would be of great help to publish a table of old versus new "names" and to explain the principles. It was decided to submit this approach as a proposal to the SSC before a report was made and ask for more general support and approval of this effort.

**Quality control of measurements of genetic polymorphisms**

Dr. M. de Maat, on behalf of the working group mentioned above, summarized measures that should be taken to assure quality of genetic methods. Measures are different for different methods. Publication of quality control techniques that are known to expert laboratories might be useful to support the dissemination of genetic methods to many new laboratories. It was decided that Dr. de Maat would arrange a collaborative study, including distribution of samples among interested laboratories, to document the magnitude of the problem. This will be reported next year.

**Items for 1998**

In addition to what has been mentioned above, it was suggested to pay attention to standardization of D-dimer and to explore the developments around TAFI.
Hemostasis and Malignancy

Chair: M. Levine, Canada
Co-Chairs: A. Falanga, Italy; A.K. Kakkar, UK; F. Rickles, USA

1. Tumour Cell and Vascular Endothelial Cell Interaction

Dr. Clazardin reviewed mechanisms of angiogenesis in tumour growth and dissemination. This included leukocyte adhesion to endothelial cells and migration, angiogenic factors regulating adhesion of leukocytes to tumour endothelium, and tumour cell interaction with the endothelium.

Dr. Giavazzi discussed assays that can be used to study angiogenesis experimentally. She presented two examples of how new, emerging antineoplastic agents have been studied in such systems (paclitaxel, batimastat). Future studies could perhaps identify those patients who might benefit from anti-angiogenic therapy.

2. Work-in-Progress

Management of Thrombosis in Cancer Patients

a. Dr. Falanga discussed tests of hemostatic markers that predict for thrombosis. There is still a paucity of well-designed prospective studies in this area.

b. Dr. Piccioli provided an update on the progress of the SOMIT study. Patients with idiopathic DVT are randomized to an extensive investigation for underlying cancer or not. Six Italian centres are participating and 122 patients have been randomized to date. The target sample size is 400.

c. Dr. Tempelhoff reviewed the data on the risk of thrombosis in cancer patients receiving chemotherapy. He is planning a trial in which breast cancer patients will be randomized to low molecular weight heparin or placebo. These patients will have operable disease (T1-4, N0-2, M0). The outcomes are thromboembolism, bleeding, survival. Dr. Levine pointed out that at the recent American Society of Clinical Oncology meeting in Denver, a number of adjuvant breast cancer trials reported rates of thromboembolism. He indicated that there is still much research to be done to learn about rates of thrombosis in sites other than breast cancer, which drugs are thrombogenic, mechanisms of thrombosis and whether such patients should be treated prophylactically.

d. Dr. Kakker discussed the risk of post-operative thrombosis in cancer patients. There are still many unanswered questions; e.g., duration of prophylaxis, and whether post-operative prophylaxis with LMWH can reduce cancer patients' mortality. He is planning a trial in colorectal cancer patients comparing mechanical with extended pharmacological prophylaxis. The outcome is mortality.

e. Dr. Levine discussed the results of three recent trials of LMWH at home compared with standard heparin by IV infusion in hospital (Canadian, Tasman, Columbus trials). The rates of recurrent thromboembolism were presented in
cancer and non-cancer patients. There were over 250 patients treated effectively with home LMWH. There was no difference between LMWH and standard heparin. The rates of recurrence were three-fold higher in cancer versus non-cancer patients.

f. Dr. Kakker presented an update on the FAMOUS trial. Patients with advanced cancer are randomized to fragmin, LMWH or placebo. The primary outcome is survival. The target sample size is 600. An interim safety analysis has been performed and the trial continues recruitment. The trial is presently multi-center in Britain. The plan is to extend it to North America.

Models of Tumour Metastasis

Dr. Rickles presented data on the relationship between tissue factor, angiogenesis and tumour cell growth. In experimental models there is a relationship between vascular endothelial growth factors and tissue factor.

Dr. Poggi discussed the antitumour and anticoagulant effects of heparin and new semi-synthetic sulfaminoheparinsulfates in murine models. She also discussed fibrinolysis in PA-1 transgenic mice and UPA knock-out mice.

Registry of Trials

Dr. Zacharski provided an update on the registry of trials of coagulation-reactive drugs in cancer. He noted the negative result of ASA on colon cancer in the Physician's Health System.

Dr. Angnelli communicated to Dr. Levine information on an ongoing, multi-center Italian trial in which patients undergoing cancer surgery are randomized to dermatan sulfate or standard heparin. Patients undergo venography at eight days.

Cancer Procoagulant (CP)

Dr. Gordon provided new information on immuno-histochemistry and flow cytometry of CP.

Acute Promyelocytic Leukemia

Dr. Rickles provided laboratory data from Intergroup Study 0129. The lab study was a companion to the main trial which compared chemotherapy with A1-transretinoic acid (ATRA). A variety of activation markers were measured in 29 patients. D-dimer, F1+2, and TAT decreased over 30 days in both groups. These patients did not have increased levels of fibrinolytic activation markers. The messenger for tissue factor dropped by Day 15 and more so in the ATRA group. ATRA increased IL-1 β gene expression.

Meeting of Executives of Subcommittee
7. The subcommittee's major focus over the last several years has been to increase the profile of the problem of thrombosis in the cancer patient, particularly amongst oncologists, i.e., the promotion of a wider discussion. To this end, Drs. Levine, Rickles, and Kakkar gave an educational session at the recent ASCO meetings in Denver. The sessions were extremely well-attended and a manuscript on the subject was published in the ASCO educational book. The subcommittee will continue its goal of expanding discussion of thrombosis in the cancer patient to a wider audience.

8. The subcommittee recognized that there is a need for a clear discussion on gaps in knowledge of issues related to management of thrombosis in the cancer patient and similarly to develop practice guidelines where there is sufficient evidence, e.g., surgical prophylaxis, duration of prophylaxis (primary and secondary). The subcommittee agreed that we should write at least one article on these issues for *Thrombosis and Haemostasis*.

9. The subcommittee also will continue to maintain a registry of trials of antithrombotic agents in cancer. We also agreed to continue to publicize ongoing trials.
Lupus Anticoagulant/Phospholipid-Dependent Antibodies

Chair: T. Barbui, Italy
Co-Chairs: J. Brandt, USA; S. Machin, UK; R. Roubey, USA; I. Scharrer, Germany; D. Triplett, USA

Attendance: approximately 325

The Subcommittee Meeting consisted of three parts:

1. Diagnosis of Lupus Anticoagulants - Antiphospholipid Antibodies
2. Clinical Aspects and Treatment of the Antiphospholipid Syndrome
3. Proposals for International Collaboration

Part 1

Prof. D. A. TRIPLETT updated the methodology for the diagnosis of lupus anticoagulants. Major points of discussion were the heterogeneity of the lupus anticoagulants and the lack of nomenclature and classification (which should take into account their differences in antigenic targets, association with thrombosis, underlying disease and types of laboratory tests employed for their detection). The laboratory methodology for the detection of lupus anticoagulants is a major task of the Subcommittee, which has repeatedly proposed recommendations (last time in 1995). Triplett underlined the current methodological problems: the failure to comply with the recommendations; the simplification of diagnosis (for example, by adopting integrated systems); the presence of pseudo-factor deficiencies and that of true factor deficiencies; the absence of reference standards and materials; the need to quantify the titer of the lupus anticoagulants. He reviewed the ongoing clinical trials (Italian Registry and WAPS) and proposed, as future work of the Subcommittee, the identification of physiopathological mechanisms of thrombosis, the development of reference standards, the quantification of the inhibitors and the development of tests (or combination of tests) able to predict the risk of thrombosis.

Prof. R. A. S. ROUBEY updated the current methodology for the detection of anticardiolipin (aCL) and anti-β2-glycoprotein I (αβ2-GPI) antibodies by immunoassays. First, he gave the general principles for the applicability of enzyme immunoassays to aCL and αβ2-GPI antibodies: the technical evaluation should consider precision, accuracy, analytical sensitivity and specificity (cross-reactivity), robustness and non-specific binding. The clinical evaluation should take into account: normal range diagnostic sensitivity, specificity and predictive values and also ROC curves. Roubey emphasized the importance of the autoantibody characteristics, such as the antigenic specificity (β2-GPI, prothrombin, other proteins and the role of phospholipids), titer, isotype, subclass, affinity, and avidity. The assessment of the immunoassays could be phospholipid-based (conventional or modified cardiolipin ELISA) or protein-based (αβ2-GPI ELISA or immunoblot). Other immunoassays could be developed for measuring anti-prothrombin antibodies. In conclusion, he gave the recommendations and suggested the future directions and the role of the Subcommittee.
Prof. P. de GROOT reported on a large clinical study performed in almost 200 SLE-positive patients that assessed the association between thromboembolic events and antiphospholipid antibodies measured either via immunoassays (aCL, aβ2-GPI and anti-prothrombin antibodies) or via coagulation tests (lupus anticoagulants). Only the presence of lupus anticoagulants was statistically associated with both venous and arterial thrombosis. Adsorption experiments were performed to investigate the contribution of aβ2-GPI and anti-prothrombin antibodies to the expression of the lupus anticoagulant activity. De Groot concluded that there is no direct clinical need to use ELISAs to detect aβ2-GPI and anti-prothrombin antibodies and that, in a large majority of patients, lupus anticoagulant activity is caused by a combination of antibodies with different specificities.

Prof. J. ARNOUT discussed the pathophysiological mechanisms of antiphospholipid antibodies. Monoclonal antibodies (MoAbs) were raised against β2-GPI and their effect on the binding of β2-GPI to a phospholipid surface was studied by real-time biospecific interaction analysis. MoAbs (and their Fab2 fragments) provided with phospholipid-dependent anticoagulant activity were able to form stable bivalent complexes on the surface; conversely, MoAbs lacking this property failed to stabilize the binding. Arnout hypothesized that this might occur also on a partially activated physiological surface, in this way potentiating cell activation via the Fc receptor.

Prof. C. N. CHESTERMAN discussed the effect of isolated IgG with lupus anticoagulant activity on thrombin generation under flow conditions. Patients' IgG was able to increase the generation of thrombin 1.2 to 5.6-fold over controls. Opposite results were obtained under static conditions. IgG with lupus anticoagulant activity was also able to protect FVa from inactivation by aPC. Patients' IgG facilitated the interaction of prothrombin to the phospholipid surface in flow but not in static conditions. This may have important implications in shifting the balance toward thrombosis in vivo.

Part 2

Dr. A. TRIPODI discussed the problems regarding the monitoring of oral anticoagulation by means of the INR system in patients with aPL antibodies. He proposed a multicenter collaboration to investigate the issue of laboratory control of oral anticoagulation therapy in patients with aPL antibodies. Plasmas from patients with and without oral anticoagulation will be collected in different centers and the INR will be evaluated centrally with commercial reagents previously calibrated against the International Reference Preparation for thromboplastin to determine the ISI. Results will be analyzed to assess the extent of interference of aPL antibodies on PT-INR and to identify the reagents more likely to be affected.

Prof. S. MACHIN discussed current knowledge on the treatment of thrombosis and reported on the ongoing clinical trials. He defined four different types of aPL-positive patients who could be enrolled in appropriate clinical studies: 1. patients without thrombosis, to establish the possible role of primary prophylaxis and the best treatment of high-risk situations; 2. patients with a single thrombotic event, for whom the duration and intensity of oral anticoagulation has to be defined, also in consideration of possible concomitant risk factors (i.e., FV Leiden mutation); 3. patients with recurrent thrombosis despite long-term, high-dose warfarin treatment (although
their number is small, the clinical impact is very high); 4. patients with cerebrovascular thrombosis (a prospective trial is ongoing). He concluded by suggesting that an International Registry with data of ongoing clinical trials be reviewed at the annual SSC meeting.

Prof. M. GREAVES provided an overview of the current knowledge on the pathophysiology of recurrent miscarriages, updated the therapeutic approaches, and discussed the risk-benefits of steroids, aspirin, heparin alone or in combination. Based on the results of a recent clinical trial, the combination aspirin/heparin (5,000 x twice daily) appears associated with a better pregnancy outcome. He reported about a randomized trial (aspirin vs. low molecular weight heparin) which is currently in progress at the Liverpool Women's Hospital. Finally, among the questions to be solved in the future, he underlined: the need to have predictive laboratory tests; the necessity to confirm the advantage of heparin/aspirin over the other treatments; the role for low-molecular weight heparins; the impact for short- and long-term side effects of heparin therapy in the mother; the optimal dose and duration of treatment; and the possible role of intravenous immunoglobulins.

Part 3

Prof. F. WISLOFF discussed an integrated coagulation system that can be used in patients with lupus anticoagulants, which incorporates in a single assay the screening, mixing, and confirmatory procedures. This system has been proposed for the aPTT and the dRVVT. Wisløff proposed to investigate the aPTT-based system in the setting of the forthcoming ISLA-5, with the aim to define the cut-off between normal and pathological (97.5 vs. 99th percentile) and to serve as a reference for semi-quantification of lupus anticoagulants.

Prof. M. C. BOFFA summarized the first meeting of the European Forum on aPL antibodies. Three projects were presented, standardization of aPL ELISA, standardization of αβ2-GPI antibodies, and registry of cases of familial antiphospholipid syndrome. She summarized the results of the questionnaire sent by A. Tincani to 29 centers to investigate the current methodology employed for the detection of aPL antibodies.

Dr. G. FINAZZI provided an update of the WAPS study, the aim of which is to investigate long-term, high-dose warfarin treatment in the secondary prevention of arterial and venous thromboembolism. The trial is due to start with patient enrollment in July 1997.

A booklet with the reports of each presentation was distributed during the Subcommittee meeting.
Perinatal/Pediatric Hemostasis

Chair: M. Manco-Johnson, USA
Co-Chairs: M. Andrew, Canada; M. Hellgren, Sweden; R. von Kries, Germany; A. Sutor, Germany

1. Neonatal Alloimmune Thrombocytopenia

Dr. Jim Bussell, USA, presented recommendations regarding which infants should be evaluated for NAIT and which laboratory assays are appropriate to assign the diagnosis. The recommendations incorporate platelet count, family history and clinical assessment of the infant. Testing will consider the racial and ethnic background of the parents. The subcommittee agreed that the recommendations be adopted as suggested by Dr. Bussell and further recommended publication in a pediatric journal as the targeted audience includes neonatalogists and general pediatricians.

2. Idiopathic Thrombocytopenia Purpura

Dr. Antony Sutor, Germany, discussed the diagnosis of childhood ITP with emphasis upon inaccuracy in automated methods for platelet counting and pitfalls in assignment of diagnosis based solely upon platelet count. In order to evaluate the recent American Society of Hematology clinical practice guidelines for ITP, it was decided that data is needed relating outcome of childhood ITP (i.e., intracranial hemorrhage) to platelet count. Dr. Sutor and Dr. Bussell were assigned to establish a European/North American registry of cases of intracranial hemorrhage in children with both acute and chronic ITP.

3. Evaluation of Children with Thrombosis

Laboratory (U. Nowak-Gottl, Germany) and clinical evaluation (M. Manco-Johnson, USA) of the neonate and child with thrombosis were discussed. Dr. Nowak-Gottl proposed a three-tiered algorithm for the laboratory evaluation of children with thrombosis. The primary evaluation should include testing for elevated lipoprotein (a), hyperhomocysteinuria, deficiencies of AT-III, protein C, and Protein S and evidence for the lupus anticoagulant (APTT and ACA). Individuals with a history of thrombosis, negative first-tier studies and a positive family history for early-onset thrombosis are evaluated further for dysfibrinogenemia, hypo/dysplasminogenemia, heparin cofactor II, HRGP, hyperlipidemia, and deficiencies of factor XII or factor V. Children with symptomatic thrombosis found abnormal only for factor V Leiden are further assessed for defects in fibrinolysis including TPA antigen and activity, PAI-1 activity and release with stasis. Evaluations include an initial evaluation at the time of thrombosis, follow-up at 3 to 6 months or after discontinuation of oral anticoagulation, and confirmatory family studies. Assay methodology was discussed. Dr. Manco-Johnson discussed necessary clinical methodology and documentation including determinations of thrombosis diagnosis, site, extent, underlying medical conditions and risk factors, treatment and outcome. The subcommittee suggested starting with diagnostic and outcome criteria. The
two sets of recommendations will be developed for publication as recommendations of the SSC based upon prevailing best practice.

4. Lupus Anticoagulants in Children

Dr. Christof Male, Austria, presented long-term outcome data of 95 Austrian children with the lupus anticoagulant evaluated between 1969 and 1996. The data suggest that children manifest a higher rate of bleeding associated with low activities of prothrombin as compared with adults. Clinically significant abnormalities were determined in children who presented with bleeding or thrombosis, not in routine screening. Dr. Male suggested a multi-center collaboration to increase the data base and the subcommittee supported this recommendation.

5. Registries

Dr. Maureen Andrew, Canada, reported results of the Canadian and international registries for neonatal and pediatric thromboses since 1990. The benefits and limitations of registries were discussed. Registries have been useful to generate baseline data needed to plan prospective randomized clinical trials.

6. Catheter-related Thrombosis

Dr. Eric Grabowski, USA, discussed various issues contributing to thrombotic risk of catheters including placement, duration, size match to vessel, wall thickness, biomaterial, perfusate, and local flow dynamics. Dr. Grabowski recommended that initial attempts to study catheter-related thromboses should focus on placement and duration. Basic research efforts continue to be focused on the other issues.

7. Pregnancy-related Thrombosis in Women with Genetic Thrombophilia

Drs. Conard (France) and Walker (Scotland) discussed a clinical approach to evaluation, therapy and thrombosis prevention in women with genetic thrombophilia. They requested another year to work with Dr. Helgren in definition of issues before developing a formal subcommittee report.
Plasma Coagulation Inhibitors

Chairperson: T. Koide (Japan)
Co-chairpersons: M. Aiach (France), R.M. Bertina (The Netherlands),
F.C. Church (U.S.A), B. Dahlback (Sweden), H. Kato (Japan)
and D.Lane (U.K.)

1. The Subcommittee completed two activities in the past year which resulted in Official
Communications. One is a database of mutations of antithrombin published in
Thrombosis and Haemostasis 77, 197-211, as "Antithrombin Mutation Database 2nd
(1997) Update." The other, "Protein S Deficiency: A Database of Mutations," is currently
in press in Thrombosis and Haemostasis.

2. We had a full-day meeting on Saturday with about 450 attendees and the meeting room
was always full to overflowing. The meeting adjourned at 4:40 p.m.

3. This year's meeting consisted of six sessions and a total of 17 papers were presented.

The contents of the session are as follows:

Protein S Deficiency

Six papers were presented and the first two were about the assay of free protein S in
plasma. The next four papers were on the genetic and phenotypic characterizations of
type I and type III protein S deficiency, paying attention to their nomenclature and
classification, in particular.

Dr. B. Dahlback (Sweden) introduced a new method of assay of free protein S that is fast,
reproducible and highly specific for free protein S. The principle of this method is called
ELSA (Enzyme-linked Ligand Sorbent Assay), which utilizes C4BP as immobilized
ligand for catching free protein S in plasma.

Next, Dr. J. Amiral (France) reported the results of the measurement of free and total
protein S and protein S activity in 520 healthy individuals. He emphasized that
cholesterol and BMI (body mass index) are major parameters affecting the total protein S
assay, that gender and cholesterol are parameters affecting the free protein S assay, and
that gender and BMI are parameters affecting protein S activity. He also emphasized that
the lower threshold for the diagnosis of hereditary protein S deficiency should be
carefully determined in subpopulations of normal subjects which include males, females,
and females using oral contraceptives.

The subsequent four papers dealt with protein S gene mutations.

Dr. R.E. Simmonds (U.K.) examined a large protein S-deficient kindred (122 germline
individuals including 44 affected) and identified Gly295 to Val mutation in three family
members. He concluded that the type I (low total protein S antigen and low free protein S
antigen) and type III (normal total protein S antigen and low free protein S antigen)
protein S deficiencies are phenotypic variants of the same genetic disorder and arose because of an age-related increase of total protein S antigen levels.

Dr. T. Yamazaki (Japan) also issued the same conclusion from a study on two protein S deficient families in Japan.

Dr. N. Sala (Spain) also reported results of the genetic analysis of families with type I and/or type III protein A deficiency which demonstrated the complexity underlying type III deficiency. It concluded that while allelic heterogeneity in the protein S (PROS1) gene is the main cause of type I protein S deficiency, type III or free protein S deficiency is likely to be a genetically heterogeneous or complex disease. Free protein S deficiency results either from a mutation in a single major gene like PROS1, or it results from the interaction of different factors, among which the protein S Heerlen allele seems to play a role.

In the last paper, Dr. R. Bertina (Netherlands) discussed protein S Heerlen allele. He concluded that, in spite of reduced levels of free protein S in individuals with protein S Heerlen allele, this allele is not associated with a risk factor for thrombosis. At the end of the session, the subcommittee chairman proposed to organize a new working party on protein S deficiency which would concern assay of free protein S and nomenclature of type I and type III deficiencies.

Protein C Deficiency

Dr. R.A. Marlar stated that a report of the Working Party on the Clinical Aspects and Treatment of Homozygous Protein C and Protein S Deficiencies is being prepared for submission to *Thrombosis and Haemostasis* as an official SSC communication.

Antithrombin

Dr. E. Gray (U.K.) reported the result of the Collaborative Study for the Second International Standard for Antithrombin Concentrate. An international collaborative study including 18 laboratories in ten countries was organized. The proposed Second International Standard, 96/520, was calibrated against the First International Standard for Antithrombin, Concentrate, 88/548, and also compared against the Second International Standard for Antithrombin, Plasma, 93/768, by both functional assays and antigen methods. As a result, based on the means of all assays against the First International Standard for Antithrombin, Concentrate, the overall respective functional and antigenic potencies for the candidate preparation, 96/520, were shown to be 4.7 IU/ampoule and 5.1 IU/ampoule. This result will be published soon as an official publication of the subcommittee.

Dr. S.C. Bock (U.S.) reported that antithrombin-beta, the quantitatively minor isoform in blood, may account for a substantial portion of antithrombin activity in the vessel wall.
Dr. V. Picard (France) reviewed a database of anti-serpin antibodies. He suggested that anti-serpin antibodies are particularly useful in structure-function studies of serpins since they can specifically react with a given conformation, showing several examples of monoclonal antibodies against either antithrombin, C1-inhibitor or PAI-1.

**Thrombomodulin gene mutations**

Thrombomodulin (TM) is an integral endothelial cell membrane protein that functions as a cofactor in the thrombin-mediated activation of the protein C anticoagulant pathway. It has been suggested that an impaired TM cofactor function also could constitute a pro-thrombotic abnormality leading to thromboembolic disease. Dr. A.K. Ohlin (Sweden) presented the data of TM gene mutation in a patient with venous thromboembolic disease and showed that a defect in the TM gene leads to familial thrombophilia. Dr. H. Ireland (U.K.) also discussed TM gene mutations associated with myocardial infarction and suggested that mutations in the promoter region of the thrombomodulin gene may constitute a risk for arterial thrombosis.

**TFPI (Tissue Factor Pathway Inhibitor)**

Two papers on TFPI were presented. First, Dr. S.P. Bajaj (U.S.) discussed correlation of the plasma levels of TFPI with various diseases. TFPI is present as free form and lipoprotein-associated form in plasma and also as endothelial cell-associated form on vascular walls. Dr. H. Kato (Japan) introduced the newly developed EIA system which uses polyclonal and monoclonal antibodies against recombinant TFPI to measure free form TFPI and total TFPI in plasma. He also compared two commercially available kits for the measurement of TFPI now available from Kaketsuken in Japan and from American Diagnostic Corporation. He showed that total TFPI was highly correlated ($r=0.87$) between the two kits; however, the correlation of free form TFPI was not correlated well between the two kits ($r=0.60$).

**APC-Resistance**

APC-resistance has been discussed at the last three meetings of the subcommittee; therefore, at this year's meeting only three papers were presented. The final one by Dr. A. Tripodi was a summary of the past discussion and a proposal for a Working Party on the Standardization of the APC-Resistance Test. At the end, the chairman suggested possible members of the working party. The preceding presentations were "Cost-benefit analysis for screening of APC-resistance" by Dr. W. Schramm (Germany) and "Factor V 506Q: Prevalence, thrombotic risk and risk modifiers" by Dr. J.P. Miletich (U.S.)

Dr. Schramm discussed the risk of venous thromboembolic events in oral contraceptive users with and without APC-resistance. He showed that the incidence was 17.3 and 1.8, respectively, per 10,000 person-year, whereas, the incidence of venous thromboembolic events in oral contraceptive non-users with and without APC-resistance were 7.2 and 1.6, respectively, per 10,000 person-year. From this survey, he suggested that although testing
of new oral contraceptive users, with and without exclusion by family history of venous thromboembolic events, seems to be less cost-effective than previously reported, screening does seem to be a rational use of scarce health care resources.

Dr. Miletich discussed prevalence, thrombotic risk and risk modifiers of factor V 506Q allele, which is the only genetically evidenced cause for APC-resistance, from a huge survey including 2,312 men from the Physicians' Health Study and 2,439 women from the Women's Health Study. From the Physicians' Health Study, he concluded that heterozygosity for the mutated allele does not detectably alter the probability of heart attack or stroke but does increase the chance for first-event venous thromboembolism about four-fold. He also showed that the mutated allele was found with similar frequency among men and women but is significantly different among ethnic groups, i.e., the carrier frequency was 5.3%, 2.2%, 1.2%, 0.5% and 1.3% in Caucasian, Hispanic, African, Asian, and Native Americans, respectively. Finally, he surprised the attendees by showing that the calculated number of heterozygous carriers is estimated to be more than 11 million among Americans and more than 423,000 are likely to be women who use oral contraceptives.

4. Two new working parties were organized during the meeting:
   a. Working Party on the Standardization of the APC-Resistance Test, APC-Resistance Assay Method, Expression of the Results, Diagnosis of Factor V Leiden, and Other Causes of APC-Resistance
   b. Working Party on Protein S Deficiency: Assay Method of Free Protein S in Plasma and Nomenclature of Types I and III

5. The following reports will be issued soon as official SSC communications of the subcommittee:
Platelet Immunology

Chair: C. Kaplan, France
Co-Chairs: R. Aster, USA; D. Beardsley, USA; P. Bechtold, Switzerland;
T. Kunicki, USA

This session was devoted to two main topics: alloimmunization and autoimmunity. Drug-induced thrombocytopenia was discussed along with heparin-induced thrombocytopenia.

ALLOIMMUNIZATION

One major point of interest concerns the laboratory diagnosis of alloimmunization, i.e., identification of alloantibodies. It was shown that an anti HPA-1a antibody collected from a pool of plasma donations can be distributed as a standard. The specificity was checked by the platelet serology quality working party. Huge differences between centers and methodology were observed concerning the dilution, but after four years of storage this antibody can still be used as a standard with dilution 1/2. The next point is to obtain a panel of cell lines for detection of the most frequent antibodies. (P. Metcalfe)

Concerning human platelet antigens, they have been identified on GPI\(\alpha\), GPI\(\beta\)b, GPIIb and GPIIIa. Most of the variants of GPIIIa are resulting from point mutation. (S. Santoso)

An agreement on nomenclature must be reached in conjunction with the working party of the ISBT and be useful not only for molecular biologists but also for physicians and blood transfusion centers.

FETAL/NEONATAL THROMBOCYTOPENIA

Fetal/neonatal thrombocytopenia can be suspected during pregnancy because of maternal or sibling history or discovered at birth.

A retrospective study has shown that an important percentage of maternal thrombocytopenia discovered during pregnancy was autoimmune in origin. The difficulty to differentiate AITP from incidental thrombocytopenia when thrombocytopenia is first discovered during pregnancy has been underlined. It could be of interest to have a maternal follow-up after delivery for diagnosis. (N. Ajzenberg et al.)

In an attempt to define the frequency of neonatal thrombocytopenia in an unselected population of newborns and the contribution of immune etiologies, a multicenter prospective study was conducted in Paris. The frequency of neonatal thrombocytopenia was 0.9% and the frequency of ascertained immune thrombocytopenia was 0.3%. This finding is of importance for monitoring thrombocytopenic newborns and management of their mothers and future pregnancies. (M. Dreyfus et al.)

Diagnosis of neonatal thrombocytopenia: What maternal parameters must be considered? Which infants must be examined? What about laboratory testing?
At the moment, severe neonatal thrombocytopenia, intracerebral hemorrhage in a thrombocytopenic neonate, unexplained thrombocytopenia, and familial transient neonatal thrombocytopenia must be considered for testing. Laboratory diagnosis requires parental platelet typing and identification of maternal antibody.

Considering fetal diagnosis and therapy, fetal blood sampling must be performed in an experienced center; the therapy is still under discussion. (H. Kroll, J. Bussel, C. Kaplan)

In regard to the standardization for the diagnosis of allo- and autoimmunization in fetuses/newborns, recommendations for therapy and management of future pregnancies will be formulated by the subcommittee.

**Autoimmunity**

**Cytokine profiles in ITP**

A brief overview of cytokines in autoimmune thrombocytopenia was presented. The theoretical basis of pro-inflammatory cytokines produced in the settings of the normal response to therapy and the abnormal response of chronic autoimmune processes such as diabetes mellitus and rheumatoid arthritis was discussed.

The results of serial screening of ITP patients at onset of disease (ITP) and, over time, measuring IL-4, IF\(\gamma\), IL-1\(\beta\), IL-2 production after stimulation of PBMC with PHA or GPlIb/IIa was presented. At diagnosis, patients with acute ITP have very low IL-4 level and marked reduction in IL-4 production on PHA stimulation (p<0.001), suggesting excess IL-1\(\beta\). Increased IL-1\(\beta\) production was observed in PBMC stimulated with GPlIb-IIIa in patients with IgG anti-GPlIb/IIa antibody, but not in the patients with anti-GPlIb/IIa IgM alone. As IL-1\(\beta\) is a potent inhibitor of IL-4, we propose that increased production of this cytokine may add to the chronicity of ITP in these patients. (D. Nugent)

**Integrin autoantigens in ITP**

Data were presented on the effect of calcium chelation with EDTA on the B cell epitope structure of GPlIb/IIa. The majority of serum samples from 25 ITP patients with anti-GPlIb/IIa antibodies were not reactive with GPlIb/IIa modified with EDTA, but showed clear reactivity with more native GPlIb/IIa. Phage display peptide libraries were used to identify patient-specific oligopeptide sequences recognized by serum anti-GPlIb/IIa antibodies. Rounds of phage selection with purified patient IgG allowed the identification of three oligopeptides (REKAKW, PVVWKN and [C]TGRVPLGFEDL[C]) which were specifically recognized by the serum IgG of respective patients. Sequencing alignments revealed some, but limited, sequence homology with either GPlIb or GPlIIa. Binding of serum IgG to peptides was specific as inhibition could be achieved with GPlIb/IIa but not with other irrelevant proteins. It was emphasized that the obtained oligopeptides are more likely to be mimotopes than the true B cell epitopes. Construction of cyclic peptides by addition of an amino- and carboxy-terminal cysteine (peptide 3) resulted in enhancement of reactivity. One of the three peptides was also
recognized by the serum IgG of another patient, which suggests the possibility of shared epitopes between patients. (R. McMillan)

**Alloantibodies to GPIa/IIa in spontaneous thrombocytopenia in mothers**

Two interesting cases of severe pregnancy-related thrombocytopenia were presented. PAIg (Kaplan, Paris) in both studies were negative but serum investigations demonstrated the presence of anti-HPA-5b (Bra) alloantibodies. Both women were HPA-5a5a. In neither case was thrombocytopenia present in the neonates. It was emphasized that these two cases of presumed immune-mediated thrombocytopenia indicate something about the possible pathophysiology of severe pregnancy-related thrombocytopenia. During the discussion, it was suggested that because of the expression level and post-transcriptional modification of the target antigen, the GPIa/IIa complex might possibly alter during pregnancy or, alternatively, alloantibodies with high affinity for GPIa-glutamine 505 (HPA-5b) might cross react with low affinity to GPIa-lysine 505 (HPA-5a) resulting in a possible reduced lifespan of maternal platelets. (A. Nurden)

**T-cell activation and abnormalities in children and adults with AITP**

A short introduction was given on the critical role of T cells in the immune response against a certain antigen. T-cell recognition of antigen-derived peptides in the context of HLA class II on professional antigen-presenting cells provides a signal to the B cells allowing proliferation of antigen reactive B cell clones and subsequent somatic hypermutation of the Ig variable domain genes, ultimately resulting in the production of high affinity IgG autoantibodies to platelet antigens. Considerable attempts have been made to clone specific T cells from the peripheral blood of patients with acute or chronic ITP, but to no avail. This suggests that precursor frequencies in the peripheral blood of autoreactive T cells is too low to permit cloning and that spleen mononuclear cells might be a more appropriate source. Intact allogeneic platelets were used as the antigen source to obtain T cells with GPIIb/IIIa reactivity from the splenic lymphocytes of an ITP patient with GPIIb/IIIa autoantibody. Splenic cells adherent to plastic proved to be much more efficient than non-adherent cells to obtain specific clones. A panel of T-cell clones were obtained which proliferated when stimulated with platelets and produced IFγ when chased with GPIIb/IIIa. Phenotyping of the T-cell clones indicated three distinct subsets CD3+, CD4+, CD8-; CD3+, CD4-, CD8+; and CD3+, CD4-, CD8-. The latter clones might well express a γ T cell receptor. Molecular studies on the TCR of these clones and their V gene use are underway. (J. Semple)

**From practice guidelines to case/diseasemanagement. Key issues:**

Practice guidelines are the initial step in a continuous process for improving clinical performance and health care delivery; however, a practice guideline, though not embedded in this process of diseasemanagement, still leaves a widespread variation open in medical practice and interventions and is rarely accepted and implemented by health professionals and physicians in particular. These guidelines, which are efficacy oriented and rarely based on effectiveness studies, seldom address costs and cost-effectiveness and are not able to overcome the traditional fragmentation of patient care, medical interventions, or care providers.
The case of chronic ITP in adults:

The practice guidelines on chronic ITP developed by the American Society of Hematology, published in 1996 in BLOOD and available worldwide on the Internet, are an important step in the right direction; however, the original publication states that only a few recommendations are based on firm evidence and that these recommendations should not form the basis for definitive decisions on health care policy. Additionally, no consensus was reached for some important issues relevant to patients and costs, e.g., is a bone marrow aspirate/biopsy appropriate or necessary to establish the diagnosis of chronic ITP; and should patients with platelet counts <30,000 with significant or severe bleeding be treated with glucocorticoids or IvIgG? Due to this lack of firm clinical data for evidence-based recommendations, the authors identified several priorities for future research:

Proposals:

- Development of a protocol for managing patients with chronic ITP based on the practice guidelines on chronic ITP. This protocol addresses the entire course of the disease and may be used as an effective and long-term follow-up and treatment plan for the individual patient, thereby linking general physicians, hematologists and hospital-based providers together.
- Initiation of effectiveness and cost-effectiveness studies on the management of patients with chronic ITP with special regard to:
  1. the effectiveness of glucocorticoids and IvIgG in patients with platelet counts <30,000
  2. the effectiveness of therapeutic interventions in patients with refractory disease and moderate thrombocytopenia (30,000-50,000) but without bleeding compared to no treatment as reference regimen (P. Berchtold)

DRUG-INDUCED THROMBOCYTOPENIA

A fluid-phase immuno-assay for anti PF4 heparin complex antibodies has been presented (B. Chong). This assay has a very low background and a higher sensitivity than PF4 ELISA kit. Weak cross reactions are observed with heparin-like anticoagulants.
Platelet Physiology

Chair: J.L. McGregor, France
Co-Chairs: E. Angles-Cano, France; M. Berndt, Australia; C. Cerletti, Italy; K. Clemetson, Switzerland; P. Newman, USA; G.C. White, USA

The platelet physiology subcommittee meeting in Florence was divided into three major parts. The two first parts ran for approximately two hours and the last part for one hour and 30 minutes. The number of people attending this subcommittee meeting was estimated to be 200.

Part I. Bioinformatics: Internet genomic registry of platelet congenital disorders (Co-Chair: J.L. McGregor, France).

The first speaker, Deborah French (USA) (dfrench@smtplink.mssm.edu), introduced the role of GPIIb/IIIa in platelet functions and the phenotyping of patients suffering from such a disorder. She did an excellent survey in putting together a registry (http://scripps.edu/bcmd) on point mutations, deletions and other defects, identified on Glanzmann thrombasthenic patients (21 individuals with GPIIb and 19 with GPIIIa defects) by their laboratory and workers in the field. She indicated the relevance of such work in prenatal diagnosis (countries where this was performed) and carrier detection. The second speaker, Alan Nurden (France), gave a very good review on the foundations that are necessary for setting up a strong registry on platelet genetic disorders. Points of importance raised by Dr. Nurden include: (1) What should go in the registry (clinical, biochemical and molecular information)? (2) Authenticity (reviewing the experimental data before insertion in the registry?) (3) Priority (what will be the attitude of journals toward the part of manuscripts made public via the Internet?) (4) New reports of previously encountered mutations, deletions, inserts, potential hotspots, etc. (5) How should the data be organized? Previous nomenclature, while useful, is outdated. (6) Who runs the database? Mount Sinai could be one of the centers with Dr. French being the database manager responsible for reviewing data and submissions. The third speaker, Kenneth Clemetson (Switzerland), gave a brief update on the possibility of setting up a registry for patients in France, Germany, Switzerland, Finland, the UK, etc., with the Bernard Soulier syndrome. He was followed by Dr. Satu Kaski (Prof. Kekomaki's laboratory, Finland) who presented data (three distinct types of mutation or deletion) on the Finnish Bernard Soulier (15 families, 22 patients in a population of five million). The fourth speaker, Dr. Dermot Kenny, presented a survey of Bernard Soulier syndrome in the Midwest region of the USA. The fifth speaker, Dr. Kenjiro Tanoue, (Japan), presented a survey of the Bernard Soulier population in Japan.

Part II. Characterization and standardization of the giant platelet syndromes (Co-Chair: Gilbert White, USA).

An attempt was made by the three speakers (Andreas Greinacher, Jim White and Paquita Nurden), after an introduction by the co-chair, to look at different giant platelet abnormalities (e.g., May-Hegglin, Fechtner syndrome, Mediterranean macrothrombocytopenia, Gray platelet syndrome, Medich inclusion disorder, Gainsville giant platelet disorder, Alport's syndrome, Montreal platelet syndrome, Chediak-Higashi syndrome) that can come under the classification of giant platelet syndrome. The co-chair stressed, in complete agreement with the speakers, that
this is an area that requires further research in molecular medicine. Phenotypes of these patients should also be made available on the Internet to allow centers to have information on these anomalies.

Part III. Standardization in signal transduction measurements in platelets and quality control (Co-Chair: Kenneth Clemetson, Switzerland).

The first speaker, Koneti Rao (USA), gave an extensive review on platelet secretion disorders. He pointed out that many platelet secretion disorders were lumped together more out of convenience than on the basis of the mechanisms underlying the dysfunction. The second speaker, Gerard Mauco (France), presented the technical basis for inositol lipid metabolism. The third speaker, Jan Akkerman (Holland), gave an excellent review on quality control of platelet functional defects.

It will be suggested to the co-chairs of the three parts of this platelet physiology subcommittee to present a report, in view of the importance of the treated subjects, for publication (following acceptance by the publication review committee) in *Thrombosis and Haemostasis*. 
Predictive Variables and Cardiovascular Disease

Chair: K. Bauer, USA
Co-Chairs: R. Hull, Canada; S. Humphries, UK; G. Lowe, UK

The number of people attending this subcommittee meeting was estimated at 450.

**Epidemiologic studies:** Dr. George Miller led off the session by presenting an overview of three major prospective epidemiologic studies. These include the ARIC (USA), PROCAM (Münster, Germany) and Second Northwick Park Heart (NPHS II) Studies (UK). Data were presented from NPHS II indicating a steep decline in coronary event rates, which has been greater than anticipated at its inception in 1989. As compared to NPHS I which was initiated in the 1970s, NPHS II includes fewer current smokers (26% versus 46%) and participants had lower mean diastolic blood pressures at entry. Plasma fibrinogen measurements were also significantly reduced in NPHS II as compared to NPHS I.

**Genetic Polymorphisms:** Dr. L. Iacoviello presented an update from the ETRO working party on "Population Genetics of Hemostatic Risk Factors for Arterial Vascular Disease." She discussed a meta-analysis of studies examining the role of the 4G/5G polymorphism in the PAI-1 promoter as a risk factor for myocardial infarction. The 4G/4G allele was associated with a 1.24-fold increased risk of coronary heart disease (CHD).

Dr. F. Rosendaal reviewed data from the Leiden Thrombophilia Study that the G20210A mutation in the prothrombin gene is a risk factor for venous thrombosis. This mutation is correlated with elevated prothrombin levels. Dr. Michael Laffan (UK) discussed data from a thrombophilia clinic population showing that elevated factor VIII:C is a risk factor for venous thrombosis. These patients also had elevated VIII:Ag levels which correlated with VIII:C. Thus far, it has not been possible to define genetic abnormalities that are associated with these elevations.

**Hyperhomocysteinemia:** Dr. M. Cattaneo (Milan) presented an overview of hyperhomocysteinemia as a risk factor for venous thrombosis. He reviewed methodologic issues related to plasma homocysteine measurements including performance of assays post-methionine loading. Dr. A. Tripodi (Milan) presented plans for a multi-center study of plasma homocysteine assays. Dr. Armando D'Angelo discussed his center's experience in evaluating hyperhomocysteinemic patients.

**Fibrinogen:** Dr. Lowe (UK) reported results from several Scottish epidemiologic studies confirming the predictive value of fibrinogen for CHD events in men and women. He commented that variability of fibrinogen measurements has decreased with use of the international fibrinogen standard. Also improved predictive power of fibrinogen measurements for CHD can be obtained using a heat nephelometry assay.

Dr. F. Haverkate (Netherlands) briefly presented results of antithrombin III, protein C, protein S, and APC resistance determinations performed by the ECAT foundation in 70 laboratories.
Parameters of Hemostatic Activation: Dr. J. Morrissey (USA) described a clot-based assay for measuring free factor VIIa levels in plasma and the results of some clinical investigations using this method. He also presented preliminary data regarding potential explanations for a ten-fold difference in normal levels between the clot-based assay and an immunoenzymatic assay reported by Philippou and Lane.

Inflammation and Hemostatic Variables: Dr. R. Tracy (USA) and F. Haverkate reviewed the topic of inflammation as it relates to hemostatic variables and CHD risk. Recent data were presented regarding elevated levels of C-reactive protein as a coronary risk factor.
von Willebrand Factor

Chair: J. E. Sadler, USA
Co-Chairs: P. Foster, USA; D. Meyer, France; F. Rodeghiero, Italy

Attendance was approximately 200.

Dr. Augusto B. Federici discussed a proposal for an international registry on acquired von Willebrand syndrome (AVWS). Based on the preliminary analysis of responses to a survey questionnaire on AVWS, mailed by Dr. Federici and Dr. Jacob Rand, creation of a Working Party on AVWS was APPROVED by voice vote.

Dr. William C. Nichols presented an update on the VWD mutation and polymorphism databases. These resources continue to be used heavily. In the absence of specific financial support, the currency and accuracy of the database is dependent on voluntary entries by the scientific community. Efforts are underway to incorporate the VWF database into other more general mutation database projects.

Dr. David L. Aronson discussed proposed labeling recommendations for plasma products for use in VWD. A survey of 38 treatment centers yielded 33 responses, 31 of which favored labeling factor VIII products for VWF content.

Dr. Mark J. Weinstein (Acting Chief, Hemostasis Laboratory, U.S. Food and Drug Administration) discussed the benefits and liabilities of using the ristocetin cofactor activity as a means of defining VWF activity and proposals for standardizing VWF-containing concentrates. These issues will be addressed in detail at a workshop on von Willebrand factor to be held at the National Institutes of Health on September 26, 1997. Members of the Working Party on VWF Assays will attend. A proposal was APPROVED by voice vote that manufacturers should be encouraged to meet the requirements to label Factor VIII products for treatment of VWD with VWF content. Measures to establish this practice will be considered by the Working Party on VWF Assays.

Dr. Dominique Meyer presented a report on the activities of a network of 32 hemostasis centers throughout France, supported by INSERM. Its aims are to improve the diagnosis of VWD, to assess the frequency of the different types of VWD, and to identify new mutations in the VWF gene.

Dr. Federici gave a progress report on the Italian Registry of VWD. A computer database was developed to collect information from 33 hemophilia centers at four-month intervals. An analysis of data for the first 637 patients from eight centers was presented.

Dr. Sadler reviewed the activities of the Subcommittee during 1995-1996 to develop consensus guidelines for the diagnosis of VWD type 1. The consensus guidelines for diagnosis of VWD type 1 were APPROVED for further evaluation through a retrospective collaborative international study by the Working Party on VWD Diagnosis.
Dr. Robert R. Montgomery chaired a session of the Working Party on VWF Assays. Dr. Trevor Barrowcliffe addressed issues concerning VWF standards, including the replacement of the current WHO plasma standard. In further discussion the topics considered were: (1) the production and distribution of VWF standards for plasma and concentrates; (2) the use of standards to evaluate assays for VWF:Ag, VWF:RCo, and multimers; and (3) the development of specific recommendations for the use of assays in diagnosis and product standardization.

Dr. Francesco Rodeghiero chaired a session of the Working Party on VWD Diagnosis. He proposed a collaborative international study to retrospectively assess the value of bleeding history and laboratory measurements in the diagnosis of VWD type 1. In response to a preliminary feasibility questionnaire, at least 20 centers around the world have agreed to participate so far. Dr. Alberto Tosetto briefly presented some methodological issues relating to the design of this study. Dr. Giancarlo Castaman discussed the relationship between phenotype and genotype based on available data.

SUMMARY OF SUBCOMMITTEE ACTIVITIES

Issues voted:

1. Creation of a Working Party on AVWS was APPROVED.
2. The proposal, that manufacturers should be encouraged to meet the requirements to label Factor VIII products for treatment of VWD with VWF content, was APPROVED by voice vote.
3. Consensus guidelines for diagnosis of VWD type 1 were APPROVED for further evaluation by the Working Party on VWD Diagnosis, through a retrospective collaborative international study.
4. A kit of VWF plasma and concentrate standards, and plasma samples from VWD subtypes and controls, will be assayed by collaborating laboratories to test the ability to diagnose VWD subtypes. This study was APPROVED by voice vote.

Completed projects:

C. Mazurier, D. Meyer

Ongoing projects:

1. Issues relating to the labeling of factor VIII products with VWF content will be addressed in detail at a workshop on von Willebrand factor to be held at the National Institutes of Health on September 26, 1997. Members of the Working Party on VWF Assays will plan to participate.
2. The Working Party on VWF Assays will address the standardization and evaluation of laboratory tests for the labeling of blood products and for the diagnosis of VWD subtypes.
3. The Working Party on VWD Diagnosis will conduct a collaborative, international retrospective study of diagnostic criteria in VWD type 1.
Drs. Blombäck and Kallner attended the annual meeting of the CEN/TC 140 in November 1997. It was held at the CEN office in Brussels and they participated as observers. Technical Committee 140 has nine Working Groups, many of which address several work items. Only those reports with importance to the SSC/ISTH are summarized here.

**Self-testing in Monitoring of Anticoagulation Treatment**

One Working Group has studied instruments for self-testing and prepared a list of elements that should be observed by manufacturers and users of such instruments. The list particularly addresses glucose meters for self-testing. We believe that the new instruments for self-monitoring of coagulation factors during oral anticoagulation treatment need special attention. A number of instruments are presently introduced to the market, using different principles both for the assay and detection. We therefore approached the chairman of the TC 140, Dr. H. Jung (Roche-Boehringer-Mannheim, DE), to initiate inclusion of these instruments. The request was not thought appropriate for TC 140, and we have therefore approached the ISO TC 212 that also deals with *in vitro* diagnostic medical devices. This TC will meet in Geneva July 7-9 and the proposal will be on the agenda. At this moment, support has been secured from the Nordic countries; Germany is likely to join after a decision June 18; and Dr. Craig Jackson might comment on the US reaction after this report.

Chairmen of the SSC’s Scientific Subcommittees, particularly of the Subcommittee on Control of Anticoagulation, should get involved in this work. Since the ISO only deals with national standardization bodies, it is necessary that these are made aware of the importance of the subject and take an active position in establishing and sponsoring the work of such an international standardization group; therefore, you should go home and find your representative to ISO TC 212 and inform him/her about the proposal. The proposal has been sent out together with a number of other documents, but an active interest for the question would improve the likelihood that a standardization of these instruments will be added to the list of work items of the TC. The formal ballot on including this topic as a work item is closed on 21 July and communicated to the national standardization bodies. In case of a positive result, the standardization bodies will be asked to appoint participants in the working group.

**Reference Measurements, Reference Materials and Traceability of Calibrators**

Another WG focuses on the metrological requirements of reference methods, materials and calibrators. Their first two standards on reference methods and materials have been accepted and the third, on the traceability of calibrators, is in its final draft. This standard will be of great importance for correct measurements. The use of calibrators is comparatively simple in the case
of inorganic and small molecules but becomes very complex for biological materials, e.g., antigens and antibodies and enzymes. The Working Group is presently struggling with the latter type of compounds. It might be useful for the SSC/ISTH to approach the convener, Dr. René Dybkaer, and offer its expertise. In particular this area is of interest for the Factor VIII and Factor IX and for the Fibrinolysis Subcommittees.

External Quality Assessment Schemes

Accreditation requires that laboratories participate in interlaboratory comparisons. Traditionally this is achieved by participating in various types of External Quality Assessment Schemes or Proficiency Testing. It is important that SSC/ISTH becomes more involved in the development of suitable interlaboratory comparison schemes. Again, it is advisable to get in touch with the CEN TC 140 convener of this work, Dr. Adam Uldall in Copenhagen or Dr. Jean-Claude Libeer, Brussels.

Single Use Containers for Venous Blood Specimen Collection

A WG has circulated a draft standard on this subject. A number of comments have been received and will be addressed by the WG. It is noteworthy that in the coagulation field, and others alike, when accurate proportions of anticoagulants (e.g., citrate) should be added increased demands on the vacuum, storage and handling of the tubes must be enforced. It is important that the national representatives to CEN 140 who are to vote on the standard and to produce comments are aware of the special demands that must be met to suffice examinations in haemostasis and, therefore, alerted to the upcoming standards.

EU Directive on in vitro Diagnostic Medical Devices

The EU Directive for the TC 140 has been worked on for several years and now seems to be finally adopted. The Directive indicates the need for vigilance and responsibility of the manufacturer to comply with specifications. This is a delicate balance between what the profession wants and what it is prepared to pay for, i.e., what the industry is prepared to do. The general spirit of the Directive has been long established, but the final document has not yet been endorsed.

Quality Management in Medical Laboratory

Accreditation of laboratories follows the ISO Guide 25 and the European Norm 45001. These are generic documents that need explanations and adjustments to be useful for the accreditation of medical laboratories. ISO has recently revised its Guide 25, but this revision has not been accepted by the ISO TC 212 which accordingly has set out to produce a standard of its own to meet the needs of the medical profession. The Committee draft, CD15189, is now being circulated for comments and will hit the national standardization bodies during the Fall 1998. If such a document is accepted, a worldwide acceptable accreditation of medical laboratories could be achieved. The draft document is scheduled to be circulated to the national standardization bodies later this year, and it is important that SSC participates in its evaluation.