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Committee Meeting**

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SCIENTIFIC SUBCOMMITTEE MINUTES
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Animal Models of Thrombotic and Hemorrhagic Disorders

Chair: Thomas Griggs, USA
Gerhard Johnson, USA; Lina Badimon, Spain

I. Review of the status of use of animal models for the study of restenosis following therapeutic interventions:

A. Dr. Griggs reviewed the clinical aspects of restenosis. Major points were:

1. Restenosis is an injury to atherosclerotic arteries.
2. Clinical restenosis is defined by angiographic criteria
3. Clinical restenosis involves geometric remodeling, thrombosis, proliferation, and matrix formation.

B. Dr. Badimon review work primarily using the atherosclerotic pig model of coronary artery injury. She emphasized the importance of thrombosis in the early stages, proliferation during the intermediate term and the late influence of matrix, a sequence similar to that seen in the human condition. She also showed data suggesting that the porcine model predicted the outcomes of clinical trials of several therapeutic agents better than did small animal models.

C. Dr. Johnson showed critical elements of small animal models. These included practical issues such as size and cost. Additionally, he highlighted key positive opportunities for use of small animals: These include:

1. Genetic control
2. Biochemical definition
3. Pharmacological correlation
4. Simulation of diseased vessels
5. Transgenic options
6. Genetic engineering

D. The subcommittee discussed the critical need for appropriate animal modeling of the stenosis problem both to study mechanisms and to define potential therapeutic benefit. There was extended discussion of the need to characterize the various models according to their best potential application. For instance, discussants agreed that small animal models are extremely helpful for understanding molecular mechanisms of proliferation but that large animal models have proven to be the best models for predicting clinical outcome of therapeutic approaches.

E. The group decided to publish the presentations of the subcommittee meeting as a review. Additionally, the subcommittee will consider using this review as a communication from the ISTH Subcommittee on Animal Models to key cardiology societies after discussion at the Florence meeting.

II. There was a demonstration of the Animal Models Registry in the internet format. A working party was appointed to develop a form for submission of new models and for establishing a mechanism for review and update of the Registry.

III. The agenda for the Florence meeting will include a program on the status, technology and science of local delivery of therapeutic agents to arteries and veins.

Biorheology

Chair: Kiell S. Sakariassen, Norway

Co-Chairs: E. Dejana, Italy; M.M. Frojmovic, Canada; D.A. Gabriel, USA; E.F. Grabowski, USA; S.R. Hanson, USA; J.F. Hubbell, USA

The Biorheology Subcommittee session was divided into three segments, Biomaterials, Complex Blood Flow; and Working Parties.

Biomaterials. Dr. K. Sakariassen reported on standardization of the effect of angiographic contrast media on thrombus formation in an ex vivo model of collagen - or tissue-factor induced thrombosis. Thrombus formation was not different in the presence of ioxaglate (ionic), iohexol or iodixanol (non-ionics). Dr. E. Grabowski presented results of a 30-patient clinical laboratory study comparing ioxaglate and iodixanol during coronary diagnostic and coronary interventions. Evidence for degranulation was observed although it was not associated with fibrinogen binding to GPIIb-IIIa. None of the contrast media enhanced platelet adhesion/aggregation in flowing whole blood in vitro.

Complex blood flow. Dr. E. Grabowski presented an overview of commercial software for computational flow analysis. Dr. P.A. Holme presented data on platelet activation and platelet microparticle formation in native blood. Significant activation and microvesicle formation were observed at wall shear rate of 10500 sec⁻¹, but not at lower shear conditions. Dr. S. Hanson reviewed published data on antithrombotic drug efficacy versus shear conditions in human and animal models of thrombus formation. It was concluded that the local flow condition at the site of thrombus formation had a profound impact on the antithrombotic efficacy.

Working parties. Dr. J. Hubbell presented the current state of characterization of flow chambers (thrombosis models) and the interpretation of results obtained with these devices. A report will be submitted for publication. Dr. G. Lowe reported on an ongoing meta-analysis of rheological variables in prediction of vascular events. A report will be submitted for publication.

Attendance was approximately 30.

Contact Activation

Chair: Alvin H. Schmaier, USA

Co-Chairs: H. Saito, Japan; P.N. Walsh, USA; W. Muller-Esterl, Germany;
B. Lammle, Switzerland

The contact activation subcommittee session was divided into three subgroups. First, the question of contact protein deficiencies and their relationship to thrombosis was discussed. Second, the area of contact protein mutations was embellished by new data on factor XI mutations. Third, novel mechanisms of contact phase activation were discussed with the intended aim of developing new assays for measurement of components of the system.

The discussion on contact protein deficiencies and their relation to thrombosis focused mostly on factor XII deficiency. Dr. W.-M. Halbmayer presented his data outlining the frequency of factor XII deficiency and venous or arterial thrombosis. Examining patients with severe deficiency, the data suggest, but by no means proves, that there is a relationship between decreased factor XII levels and thrombosis. Dr. W. Heller presented data indicating that contact factor levels are reduced prior to and during cardiopulmonary bypass. It became clear during the discussion that standardization of normal plasmas for factor XII levels as well as prekallikrein and high molecular mass kininogen ought to be performed. Dr. M. Gallimore presented his data showing that his factor XII amidolytic assay gave evaluable factor XII levels in the presence of lupus anticoagulants. Dr. P. Esnouf presented work showing that factor XII does not participate in dietary hyperlipemia and induced factor VII activation. Dr. Schmaier orchestrated an open discussion on continuing an international registry/prospective study on contact protein deficient patients and their incidence for thrombosis. This data base, which is located at the University of Michigan at Ann Arbor, is confidential with each participating investigator keeping the name and address of the patient in his or her office. It was also suggested that the subcommittee standardize control plasmas for contact proteins with the SSC standard plasma. Further, it was suggested that an international registry of contact protein mutations be organized. Severe contact protein deficient patients should have their DNA examined for possible mutations leading to these defects.

The sessions on contact protein mutations centered on new factor XI mutations. Dr. H. Perez presented her data showing two new mutations in the Jewish population of factor XI deficient patients. In particular, one was in an Askenazic Jew and another in a Separdic Jew. Dr. J. McVey present nine different mutations recognized in their laboratories in patients who are not of Jewish origin. There was no commonality of defects in the non-Jewish population of factor XI deficient patients.

The last subsession focused on novel pathways whereby contact proteins participate. The purpose of these presentations was to stimulate development of new assays to evaluate contact protein participation in disease states. Dr. P.Kr. von dem Borne presented his data showing the accelerating amplification of factor X by factor XI activation by α -thrombin. Dr. V. Gurewich presented his laboratory's work showing that the assembly of contact proteins on platelets and endothelial cells allows for a factor XII-dependent, prourokinase activation system. Dr. A. Schmaier presented their data showing that on umbilical vein endothelial cells, there is a factor

XII-independent prekallikrein activating system which is regulated by high molecular weight kininogen. Both of these latter studies show a mechanism for cellular fibrinolysis independent of tPA and fibrin.

Control of Anticoagulation

Chair: T.W. Barrowcliffe, UK

Co-ChairS: G. Agnelli, Italy; A.M.H.P. van den Besselaar, Netherlands;
B. Boneu, France; S.M. Lewis, UK; P.M. Mannucci, Italy; L. Poller, UK

The meeting was divided into three sessions: first, thrombin inhibitors; second, heparin and LMW heparin; and third, PT standardization. Approximately 130 people attended.

THROMBIN INHIBITORS

Dr. Gaffney outlined the approach previously adopted for standardization of in vitro potency measurement of hirudin, based on a simple chromogenic substrate titration assay and use of the a- thrombin international standard. It was suggested that this approach could be extended to other thrombin inhibitors.

Dr. Fareed presented data showing that a similar amidolytic titration method worked well on a variety of thrombin inhibitors, although potencies were not necessarily predictive of in vivo antithrombotic activities. In discussion Dr. Hemker pointed out that such a method may be less robust for reversible inhibitors like argatroban compared to the stronger binding hirudin which is essentially irreversible; reaction conditions would need to be defined carefully.

Dr. Nowak described the advantages of the ecarin clotting time (ECT) for patient monitoring. The ECT gives good reproducibility, a linear dose-response curve up to 5 mg/ml hirudin, and is not influenced by heparin at up to 2.5 IU/ml. In discussion it was pointed out that the method had not been subjected to an inter-laboratory collaborative study, and it was suggested that such a study could be organized by the subcommittee. The chairman agreed to look into this possibility together with the manufacturers, members of the subcommittee and other interested individuals.

HEPARIN AND LMW HEPARIN

Following discussion at the last subcommittee meeting, Dr. Gray described the strategy adopted for the establishment of reference plasmas to help with standardization of APTT and anti-Xa measurements for unfractionated heparin. Trial fills of plasma from different sources would be prepared and evaluated in a collaborative study before proceeding with large-scale preparation of materials.

Dr. Cambus, with colleagues Drs. Boneu, Hemker and Beguin, described in vitro and ex vivo studies on the new endogenous thrombin potential (ETP) method, previously reported by Dr. Hemker at the last subcommittee meeting. In vitro studies showed a good correlation with anti-IIa assays on a variety of materials and very little activity for the pentasaccharide. In an ex vivo study of a single LMW heparin, the ETP correlated better with anti-IIa than with anti-Xa assays, but ETP inhibition, unlike anti-IIa activity, persisted for 24 hours. The current method was found to be too sensitive for monitoring high-dose unfractionated or LMW heparin but modifications were being developed by Dr. Hemker for this purpose.

Dr. Giles led a discussion on the monitoring of LMW heparin. The main issues were whether to monitor, especially in high-dose treatment, and if so, when and how. Dr. Andrew considered that pediatric use of LMW heparin was likely to increase dramatically, and required different considerations for adult use - for instance, pharmacokinetics were different and children required higher doses per kg.

Dr. Samama reviewed the published information on monitoring of LMW heparin in adults. Many trials had been published without monitoring therefore it was difficult to assess its clinical value, but there was evidence from some studies that anti-Xa levels could be correlated with clinical events.

In discussion, it was felt that there was a need to reassess the review carried out by Dr. Boneu for the Subcommittee in 1993 and subsequently published. The chairman proposed a working party, consisting of Drs. Giles, Samama, Andrew, Boneu, Hemker, and Ofosu, to examine this issue and report back to the Subcommittee next year with proposals. Dr. Giles would coordinate the activities of this group and members of the Subcommittee and other interested individuals would consult with any draft proposals.

PT STANDARDIZATION (1) WHO Human Thromboplastin

Dr. Tripodi presented the results of a large multi-center study to calibrate the replacement WHO human thromboplastin. The two candidate preparations X/95 and Y/95, both human recombinant reagents, were calibrated against the three existing WHO thromboplastins (human, rabbit, bovine) in twenty laboratories. On the basis of three criteria, i.e., within laboratory CV, between laboratory CV's, and conformity to the WHO model, there was a clear preference for X/95 over Y/95. Dr. van den Besselaar presented results of preliminary stability studies indicating that both preparations showed improved stability compared with previous recombinant reagents. After brief discussion, the Subcommittee agreed by unanimous vote of the twelve members present, that preparation X/95 should be recommended to WHO as the replacement human thromboplastin reference preparation.

(2) REFERENCE PLASMAS

Dr. Barrowcliffe presented results of a multi-center study designed to calibrate a set of European Reference Plasmas (1 normal, 2 abnormal) for INR. The study was combined with that of Dr. Tripodi, using the same laboratories and the same WHO reference thromboplastins. There were some differences in INR's with the different WHO thromboplastins: the size of the discrepancies being largest for the plasma with the highest INR. In discussion, the desirability of preparing plasmas with minimal discrepancies in INR with different thromboplastins was emphasized.

Brief presentations were made by Mr. Kitchen, Dr. Houbouyan, and Dr. Moritz on the use of INR-calibrated plasmas. All agreed that such plasmas could reduce inter laboratory variability, but careful attention should be paid to the type of plasmas, coumarin patients' plasmas giving better results than artificially-depleted plasmas, their method of calibration, and their practical use in the laboratory.

Dr. Poller gave an update on the European concerted action project on anticoagulant control. Two thromboplastin reagents, one recombinant and one rabbit brain, had been prepared for large-scale field studies. The use of artificially-depleted plasmas for local ISI determination had been further investigated. Initial studies showed that a minimum of 20 of such artificially-depleted plasmas and 7 normals were required for reliable results.

In summary, the co-chairman Dr. van den Besselaar stated that the use of reference plasmas could improve inter-laboratory variability, but possibly at the expense of accuracy.

(3) CLINICAL STUDIES

Dr. Mörssdorf and Dr. Palareti gave brief presentations emphasizing the importance of relating INR data to clinical results. Dr. Palareti presented evidence for improved therapeutic quality of anticoagulant control with the more sensitive thromboplastins ($ISI < 1.2$).

(4) WHO REQUIREMENTS ON THROMBOPLASTINS AND PLASMAS

WHO had accepted the recommendation from the Subcommittee on the need for review of these guidelines, published in 1983. A small drafting group, consisting of Dr. van den Besselaar, Dr. Tripodi, and Dr. Poller, has been established by WHO to make the first draft of the review. Dr. van den Besselaar outlined the points which were being considered and called for written comments and suggestions from participants in the meeting. Draft proposals would be prepared and presented at the next Subcommittee meeting.

SUMMARY OF SUBCOMMITTEE ACTIVITIES AND FUTURE WORK

Thrombin Inhibitors: Possible collaborative study on Ecarin Clotting Time (ECT) to be investigated before the next meeting.

Heparin and LMW Heparin: (1) Reference plasmas for unfractionated heparin - ongoing project; (2) Monitoring of LMW heparin - working group to report proposals at next meeting.

PT Standardization: (1) WHO thromboplastin: The final report is to be prepared for WHO and subsequently published; (2) Reference plasmas: Recommendations on the preparation and use of reference plasmas are to be prepared by the Subcommittee, with the aim of incorporation into the WHO guidelines; (3) WHO guidelines: The drafting group is to present the proposed revision at the next meeting.

Factor VIII and Factor IX

Chair: Jorgen Ingerslev, Denmark

Co-Chair: Ernest Briet (secretary), Netherlands

1. The chairman apologized on behalf of Professor Ian Peake who was unable to be present and outlined the program for the afternoon.
2. Drs. Hill and Mannucci presented the final draft of a protocol for the surveillance of viral transmission in previously untreated patients, which includes testing for ALT and serological markers for hepatitis and HIV. PCR may be included for parvovirus B19. A manuscript will be submitted to the subcommittee for circulation and approval.
3. On behalf of Dr. E. Berntorp, Dr. Lou Aledort presented a summary of the previously presented approach for the surveillance of viral transmission by blood products in non-infected patients (NIPS) by PCR technology. A study is being carried out in Germany and Sweden. Results from this study may form the basis of a formal SSC protocol.
4. Dr. Jeanne Lusher, also on behalf of Dr. Ives Laurian, reviewed the pharmacokinetic factor VIII and factor IX study in infants.
5. Genetic basis of factor VIII inhibitors. Dr. Charles Hay presented preliminary data from UK hemophilia centers on Hla class II subclasses as well as factor VIII gene mutations in British hemophilia patients demonstrating a weak over-representation of certain subsets. Dr. Gordon Bray and Dr. Deborah Hearst presented the inhibitor cohort studies in recipients of Recombinate and Kogenate respectively. The formation of a collaborative registry on genetic aspects of inhibitor formation was encouraged.
6. Professor Alan Giles, also on behalf of Dr. Bert Verbruggen, reported on a comparison of the classical and the Nijmegen modification of the Bethesda inhibitor assay. The material appears to be ready for submission as a final report.
7. Dr. Steve Kitchen summarized data on the secondary plasma standard for factor VIII:C, IX:C, vWF:Ag and vWF: activity. This presentation will be repeated at the second meeting of the subcommittee in Dublin on Friday, June 28.
8. Professor Donna DiMichele presented the results of an extensive registry on inhibitor tolerance induction in American hemophiliacs. This report is ready for submission to the subcommittee chair.
9. Dr. Jeanne Lusher made a proposal for the collection of data on the use of porcine factor VIII in pediatric cases of hemophilia. A postal survey is being planned.
10. The meeting adjourned at 17.05.

Factor XIII

Chair: Laszlo Muszbek, Hungary

1. The chairman of the session announced that Dr. Jan McDonagh, the chairperson of the Subcommittee, could not attend the meeting, and on the request of ISTH/SSC, Dr. Muszbek, as one of the two co-chairmen of the subcommittee, organized this meeting and serves as acting chairman. Any proposal made by the subcommittee will be forwarded to Dr. McDonagh.
2. Past year activity: a recommendation for the nomenclature of blood coagulation factor XIII (FXIII) that has been put in its final form is ready for publication in *Thrombosis & Haemostasis*. The manuscript has been sent to Dr. McDonagh but, probably due to miscommunication, no further decision has been made in this matter. Dr. Muszbek will try to contact her again and, hopefully, the manuscript will reach SSC in the near future.
3. The chairman announced a few changes in the program. Two speakers, Drs. Vivien Yee and Róza Ádány, due to other engagements, could not attend the meeting. As a replacement, Dr. Bishop had an additional presentation on "FXIII replacement therapy in the treatment of inflammatory bowel disease: An animal model study."
4. Program: A. Progress report on the ETRO survey of factor XIII deficient patients. Rainer Seitz (Langen, Germany) and Alberto Tosetto (Vicenza, Italy) summarized the present stage of the survey initiated by the Factor XIII Working Party of the European Thrombosis Research Organization (ETRO) two and a half years ago. Sending a questionnaire to major European haemostasis centers and to all ETRO laboratories and publishing the questionnaire in major thrombosis haemostasis journals have resulted in the collection of data from 72 patients of 62 families. Now a second questionnaire has been formulated and mailed to all responders. The data collected so far have revealed some interesting aspects of FXIII deficiencies. The severity of this bleeding diathesis seems to vary more considerably than it was believed and no obvious relationship with the level of FXIII seems to exist. At this point, however, it was emphasized that the data obtained are based on different FXIII assays performed in different laboratories and caution in the interpretation of the results is needed. This problem underlines the need of standardization of FXIII assays and repeating the measurements on plasma samples distributed to three laboratories with special expertise on assaying FXIII. There is no common policy on the supplementation therapy of patients with severe FXIII deficiency. Some laboratories consider it absolutely essential to initiate life-long replacement therapy at the time of the diagnosis while at the other extreme some laboratories recommend replacement therapy only when bleeding complications occur or before surgical interventions. A consensus conference seems to be needed in this respect. It could also be firmly established that impaired wound healing, although frequently (30-40%) accompanying FXIII deficiency, is not an essential symptom of the disease. Interestingly, and unexpectedly, there were reports on a few heterozygous patients with mild to moderate bleeding tendency.

One of the aims of the survey was to collect samples from a relatively higher number of FXIII deficient patients for molecular genetic analysis. This work has been started, some samples have been collected and analyzed in the Department of Clinical Chemistry, University of Helsinki and

part of the results have already been published. Hanna Mikkola from Helsinki presented a comparative report on the molecular genetics of factor XIII deficiencies reviewing their own results and the data reported in the literature. Among others, an interesting case was presented in which the nature of the molecular genetic defect explains the relatively mild phenotype of a patient with severely decreased FXIII activity.

The following recommendations were made by the subcommittee:

- a. The survey is to be kept open to be able to register further patients and the establishment of a permanent registry was proposed.
- b. There was general agreement on the benefit of extending the survey beyond Europe. However, no one among the participants was willing to accept such a responsibility. Therefore, it was decided to go on with the survey in Europe and bring up this matter again at the next subcommittee meeting.
- c. To be able to reach more solid conclusion on the relationship of FXIII activity and the severity of bleeding diathesis, it was recommended to collect plasma and possibly platelet samples from the patients included in the registry and analyze them for FXIII activity and antigen in three independent laboratories (Vicenza, Langen and Debrecen).

B. At the previous subcommittee meeting the issue of establishing an international reference FXIII preparation was raised and at the present meeting Dr. Paul Bishop (Seattle, USA) presented data on the properties of recombinant cellular FXIII (FXIII A2) to decide if such a preparation could satisfy the requirements of a reference preparation. The data presented on the stability, kinetic parameters and the comparison of rFXIII with native FXIII are promising and clearly suggest the feasibility of such a move.

It was recommended that an action plan on steps to achieve this goal be worked out for the next subcommittee meeting.

C. The policy of the laboratory diagnosis of FXIII deficiency was dealt with by Dr. Muszbek (Debrecen, Hungary). It was recommended to abandon the use of the clot solubility test as the sole screening test for FXIII deficiencies because it misses part of the FXIII deficiencies. An algorithm for achieving the diagnosis was presented and agreed upon by the attendees. The issue of the poor standardization of FXIII assays was elaborated and illustrated by several examples. A preliminary evaluation of the single commercially available FXIII activity assay (Behringwerke, Germany) was presented and weaknesses and advantages of the assay were pointed out.

The need for critical, independent, multicenter evaluation of new commercially marketed FXIII assays was emphasized by the discussants. A proposal for the policy of such evaluations will be worked out by a group of experts for the next subcommittee meeting. It was recommended that the appropriate committee of the International Federation of Clinical Chemistry be contacted and that we join forces concerning the standardization and evaluation of FXIII assays.

Fibrinogen and DIC

Chair: Willem Nieuwenhuizen, The Netherlands

This year's agenda comprised the following items:

W. Nieuwenhuizen gave an update on the reference material to harmonize the currently available quantitative "D-dimer" assays. The manuscript on this subject has been drafted and sent to the co-chairman for approval. The NIBSC has agreed to vial and distribute the reference material, which will consist of a pool of patient plasmas, and will have an assigned consensus value (see last year's report). The manufacturers will be notified and invited to report in the kit inserts how their calibrators relate to the reference material.

P. Gaffney reported on the calibration of an SSC plasma with respect to fibrinogen content. The Immuno Pharmaceutical Company (Vienna) has prepared a large batch of pooled human plasma which has been dispensed into 1 ml aliquots, lyophilized and stored at -20 degrees C. The Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) has requested that this be calibrated in terms of a number of coagulation factors. Gaffney presented a summary of a collaborative study to calibrate this plasma standard in terms of fibrinogen (clottable form) using the International Standard for Plasma Fibrinogen (89/644) as a calibrator in an automated Clauss procedure. The Clauss procedure is based on the turbidity (light scattering) of plasma following the addition of thrombin. This study was conducted under the auspices of the Fibrinogen Subcommittee. Twelve laboratories were requested to assay the fibrinogen content of SSC plasma. The overall mean was 2.58 g/l with 95% confidence limits between 2.48-2.69. It was suggested that the SSC plasma standard be calibrated to contain 2.6 g/liter. It was suggested to name this material the Secondary Standard.

M. Matsuda presented a proposal for a nomenclature for abnormal fibrinogen. This nomenclature describes the mutation in amino acids and base pairs, and is more informative on the (point) mutation in the abnormal fibrinogen molecule than the currently used indication by the name of the place where the abnormality was first discovered (e.g. Tokyo II, Cedar Rapids which are identical). It was decided that this nomenclature be published as an official ISTH publication.

C. Francis presented a survey and comparison of the currently available assays for soluble fibrin. It is obvious that the problem of standardizing soluble fibrin is a major challenge. Several options are under investigation. Important information will come from the THROMBO study and the DIC Study (see below).

Drs. Taylor, (Chairman of the DIC Subcommittee) Francis and Nieuwenhuizen presented a draft protocol aimed at assessing the clinical utility of soluble fibrin assays in DIC. Several assays are currently available, which are not (or may not be) fully comparable. DIC patients will be selected on very strict and specific criteria. Twelve clinicians have agreed to participate by supplying samples. The plan is to have results available in Florence in 1997 with a final report in 1998.

Fibrinolysis

Chair: Cornelis Kluft, The Netherlands

Co-Chairs: Bernd R. Binder, Austria; Jorgen Gram, Denmark;
Dusan Keber, Slovenia; D. Strickland, USA (unable to attend).

Attendance was approximately 80.

Dr. Cornelis Kluft summarized the activities of the last two years to establish a pilot project group for coordinated standardization of materials and methods (PGM), and the actions of this PGM to define its procedures. In brief, for each selected analytical target, a working group will be formed to define criteria for specificity of method(s) to suggest how this specificity in practice can be verified and to suggest the required reference material. Further in the session this procedure was considered for a number of analytical targets.

Urokinase Antigen and Scu-PA

Dr. Bernd Binder and K. Benraad reported on the status of standardization of assays for urokinase-type plasminogen activator antigen and the single-chain form of u-PA. Assay of both plasma and tissue extracts of tumors was considered, and it was felt to be important to combine standardization in these different areas of research. It was concluded that for u-PA antigen residual, problems needed to be solved first. These problems included differences between tumor-derived and recombinant u-PA preparations in some assays; differences in quantity and composition of the extract of tissues depending upon the extraction procedure; large inter-assay variability of some methods in some laboratories; and the presence of a significant portion of u-PA antigenic material in plasma that is unidentified.

For scu-PA assay, Dr. Bernd Binder concluded that a fair agreement exists between methods about its plasma concentration, while initial problems with an interfering component in 10% of patients with coronary heart disease were practically solved. Furthermore, evidence was accumulating that assay of scu-PA is relevant for coronary heart disease.

Dr. Patrick Gaffney summarized the work carried out in 1993 to create a standard for scu-PA used for thrombolytic treatment and to formulate an activation procedure essential for measuring its biological activity. It was concluded that Drs. Binder, Benraad and Gaffney should join forces and act as a working group within the PGM frame work to establish multiple standards in relation to specific assay methods. A call for further participants was issued (respondents thus far are Drs. V. Gurewich and W. A. Gunzler).

Standards for Thrombolytic Agents

Dr. Patrick Gaffney reported that the WHO (1995 meeting ECBS-WHO) is recognizing that mutant proteins may be significantly different from the wild-type original protein and have independent properties; thus requiring a separate reference material. Reteplase (93/726) is presently under study and might be proposed this year. Also staphylocoagulase (94/718) is under

consideration. Dr. Patrick Gaffney issued a call for participants of a collaborative study to assign a value to the new DAI-I activity standard.

Antiplasmin

Dr. Bart Hennis, on behalf of Mr. Piet Meijer who could not be present, reported problems with the specificity of antiplasmin assays were particularly noted in automated versions of this method. A major determinant of the problem was identified and appeared to be the use of too high concentrations of plasmin. It was concluded that based on this work, a working group of the PGM should be formed. A call for participants was issued (respondent thus far is Dr. M. Hanss).

Histidine-Rich Glycoprotein

Dr. Bart Hennis reported on the occurrence of two molecular forms of HRG determined by the presence of a polymorphism in the population. This polymorphism results in variable glycosylation of HRG. He reported that two polyclonal antisera used in radial immunodiffusion showed a different reactivity towards both forms (ratio 1.4). It was concluded that better assays should be developed that are either insensitive to the difference or specific to the forms.

Tissue-type Plasminogen Activator

Dr. Cornelis Klufft reported on the impact of heterogeneity in the Actilyse preparation on assays. The heterogeneity concerns glycosylation in kringle 2 of t-PA which is absent in a part of the molecules. It could be shown that this heterogeneity has no impact on two commercially available antigen assays of t-PA, but a check of other methods remains necessary. It could be shown (and was known from literature) that in measurement of activity, the two forms showed a large difference. It was concluded that for t-PA activity more work is required before standardization can be successful. For t-PA antigen, it was decided to form a working group within the frame of the PGM. A call for participants was issued (respondents thus far are Drs. M. Stegnar and D.C. Rijken).

Future Activities

Next to the working groups for scu-PA, antiplasmin activity and t-PA antigen, the subcommittee members suggested the following activities: (a) to have the canceled discussion on receptors, (b) to discuss the D-dimer standardization also in this subcommittee, (c) to discuss genetic methods and nomenclature and summarize the situation about fibrinolysis components, and (d) to discuss the practical aspects of anticoagulation for fibrinolysis; notably the problem of requirement for multiple anticoagulants.

Dr. J. Gram reported on a collaborative study to evaluate suitability of the secondary coagulation standard of the SSC for fibrinolysis quantities. The conclusion was that the matrix standard is suitable for t-PA antigen, PAI-I antigen, antiplasmin and plasminogen activity assays. The next step to assign value to the standard is considered difficult and will be attempted in collaboration with Dr. Patrick Gaffney.

Lupus Anticoagulant/Phospholipid-Dependent Antibodies

Chair: Douglas A. Triplett, USA

Co-Chairs: Tiziano Barbui, Italy; John T. Brandt, USA;
Sam Machin, UK; Robert Roubey, USA; Inge Scharrer, Germany

I. Introductory Comments on Mission of LA/PL Dependent Antibodies Subcommittee

II. Methods: Lupus Anticoagulants (LA)

Dr. Brandt reviewed current "state of the art" for laboratory testing of LA. An emphasis was placed on utilizing guidelines as recommended by the subcommittee (Thrombo. Haemost. 74: 1185-1190, 1995). Also the difficult diagnostic problem of a "pseudo factor deficiency" was highlighted. Recommendations for tests used in this setting were reviewed: chromogenic assays, use of animal deficient substrates, and antigenic assay. It is important to have a logical approach to diagnosis of LA in patients with Hemophilia A and HIV infection.

Eva Marie Jacobsen reported on the possibility of false negative screening tests for LA. The Wisloff approach to an integrated test system was discussed.

Perhaps the most provocative prevention was that of Stephen Moll from Duke University. He presented data showing remarkable heterogeneity of thromboplastin response to LA. In cases of patients with prolonged baseline PT results (not on oral anticoagulants), there was variable prolongation of PT values. When these were expressed as INRs, there was an even more profound spread of data.

III. Methods: Anticardiolipin (ACA) and Anti-b2 Glycoprotein I Assay

Drs. Roubey and Matsuura extensively reviewed the use of anti-b2 Glycoprotein I assays (focus on ELISA). The variables involved in the assays were highlighted (e.g., sensitive microtiter plates, lipid-free b2 Glycoprotein I, concentration of b2 Glycoprotein I, pH etc.).

IV. Clinical Trials and Registry Reports

Professor Barbui presented the Warfarin for Prevention of Recurrent Thrombosis in the Antiphospholipid Syndrome (WAPS). In a randomized clinical trial, Dr. Hannah Cohon discussed a prospective trial to treat women during pregnancy to prevent recurrent fetal loss (low-dose aspirin (75 mg) versus low-dose aspirin and low molecular weight heparin).

V. Standards

Professor Sam Machin presented a report on progress by NIBSC to prepare a reference plasma for Lupus Anticoagulants. This reference preparation should be available by October 1996.

Perinatal/Pediatric Hemostasis

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

The agenda of the meeting was constructed with the assistance of all members of the subcommittee who were contacted in December 1995 for their suggestions. Due to the inability of Dr. Helgren to attend the 1996 subcommittee, topics that were specifically perinatal were deferred until 1997. The final agenda contained two parts; presentations on thromboembolic disease in the infant and child, and secondly on recommendations for prophylactic use of vitamin K in newborns.

Dr. Andrew introduced the topics focused on thromboembolic disease in children by summarizing the activities of the subcommittee on this problem over the past year. There was joint sponsorship of an International Children's Thrombophilia Network by the subcommittee and the Canadian Children's Thrombophilia Society. Dr. Andrew reported on the membership and accomplishments of the Network. Approximately 250 institutions world wide are part of the network. Any physician interested in the field can join the Network. A quarterly newsletter and relevant new publications in the literature are regularly mailed to the membership. Educational pamphlets on the use of oral anticoagulants, heparin and thrombolytic agents were prepared over the year and provided to the Network. Two international randomized controlled trials were funded by industry and will assess the role of low molecular weight heparin in pediatric patients.

Pediatric Thromboembolic Disease: The first presentation was by Dr. Nowak-Gottl who presented the results of a registry in Germany on thromboembolic complications in newborns. Several important issues were identified and discussed including the underlying diseases, importance of vascular catheters as insiting agents for arterial and venous thrombosis, the difficulties in performing angiographic studies in newborns and reliance on doppler/ultrasound, the presence of congenital prothrombotic disorders, and the rather inconsistent use of antithrombotic agents for both the specific agent, and duration of therapy. The discussion focused on the difficult issue of treatment, particularly the lack of information on the indications for long-term treatment. In addition, the uncertainty of the optimal way of managing children with congenital prothrombotic disorders was assessed and discussed. The second presentation was by L. Mitchell who presented a Canadian cross sectional study assessing the relationship between antiphospholipid antibodies (APLA) and thromboembolic events in children with systemic lupus erythematosus (SLE). The results clearly showed that the persistent presence of APLA are highly predictive of thrombotic events in children with SLE. The discussion focused on the short term and long-term treatment of these children with anticoagulants.

The third presentation was Dr. M. Manco-Johnson who presented case series describing the presence of APLA in a variety of childhood conditions, particularly ivaricella. She presented data describing the co-existence of acquired protein S and C deficiency. The fourth presentation was Dr. Michelle David who reviewed the world literature and the Canadian registry of children with mechanical heart valves. The Canadian registry contained approximately 100 children followed for approximately 10,000 patient years. Together the data showed that the incidence of

thrombotic events was low if adequate therapy with oral anticoagulants were used. The data did not support the routine use of an antiplatelet agent but the latter strategy could be used for children who had had events while receiving adequate treatment with oral anticoagulants. There was agreement from the subcommittee that Dr. David should prepare a position paper for publication as recommendations from the ISTH on the management of children with mechanical heart valves. The fifth presentation was by Dr. N. Schlegle and focused on the mechanisms responsible for thrombotic complications in children with nephrotic syndrome. The final presentation was by Dr. A. Michelson who provided a summary of the recently published North American guidelines for antithrombotic therapy in children. Each of the recommendations was presented in detail and the subcommittee agreed to ask Dr. Michelson to prepare a position paper for publication as recommendations from the

ISTH. Discussions for all presentations identified the urgent need for clinical trials with the goal of assessing the optimal use of antithrombotic agents in children. The need for concurrent studies assessing the relationship of congenital prothrombotic disorders to the development of thrombotic complications in children was also discussed.

Vitamin K Deficiency: Drs. von Kries and Sutor chaired this portion of the program. The position paper prepared by Dr. Sutor based on discussions at the subcommittee in 1995 was presented. The manuscript had been circulated to the entire subcommittee within the previous two months and their comments incorporated into the manuscript. There remained two problematic areas, the merits of frequent low dose vitamin K supplementation in the postnatal period compared to the use of high dose intramuscular injection and the relationship between Vitamin K supplementation and childhood cancer. Dr. M. Cornelissen presented new information on daily low dose vitamin K supplementation and Dr. Von Kries discussed the literature and his own comprehensive study describing the relationship between vitamin K deficiency and cancer. There was agreement from the subcommittee that Dr. Sutor should form a working group to finalize a position paper for publication as recommendations from the ISTH.

There was a final informal discussion of the two funded international trials assessing the role of LMWH in children with thrombotic disease. Other problems that required clinical trials to answer the questions were discussed. The plan was for frequent communications of protocols over the upcoming year and to plan for a meeting at the American Society Of Hematology in approximately 6 months.

Plasma Coagulation Inhibitors

Chair: T. Koide (Japan)

Co-Chairs: R Bertina (Netherlands), F Church (USA), S Iwanaga (Japan),
D Lane (UK), P Sandset (Norway).

S Bajaj (USA), B Dahlback (Sweden) could not attend.

1. From this year's meeting, our subcommittee included topics on tissue factor pathway inhibitor (TFPI) which have been discussed at the independent subcommittee meeting. Accordingly, Drs. Per M. Sandset and S. Paul Bajaj joined us as new co-chairs. In addition, Dr. Frank C. Church joined us as a new co-chair specializing in a serpins section.

2. We completed two activities in the past year. One is a database of mutations of protein C, and this has been already published in *Thromb. Haemost.* as "Protein C Deficiency: A Database of Mutations, 1995 Update." The other one is an inter-laboratory study on the assay of tissue factor pathway inhibitor.

3. We had about 250 attendees and the meeting room was always full.

4. This annual meeting of plasma coagulation inhibitors consisted of two parts and a total of 12 papers have been presented. The first part was APC-Resistance Test and Related Subjects. In this session, eight papers were presented, and five of them were focused on the APC resistance test to establish a standard of the assay and the diagnosis in the near future. S. Rosén (Sweden) and J. J. Jorquera (Spain) reported a modified APC resistance assay including factor V deficiency plasma with a heparin antagonist (V-DEF Plasma) and showed advantages and limitations of the modified APC-resistance test. A. Tripodi (Italy) reported the results of the multicenter study on the performance of different home-made and commercial APC-resistance methods to detect factor V mutation and suggested that the best discrimination could be observed not for the APTT assay but for the method based on factor Xa-clotting assay. M. M. Samama (France) reported the usefulness of the modified APC resistance test in the diagnosis of thrombophilia with factor V mutation and also suggested the superiority of factor Xa-initiated clotting assay. Lastly, J. P. Miletich (USA) made a very interesting report on APC-resistance in self-described ethnic groups in the US. He and his co-workers tested 2,438 post-menopausal US women and found a big imbalance in frequency of the factor V Leiden allele which is the major genetic cause for the APC-resistance. He showed that it is most common in women reporting as White, followed by Hispanic, Black, and Asian/Pacific Islander at significantly lower frequencies. In the latter part of this session, three papers were presented. R.A. Marlar (USA) and M. David (Canada) gave the initial report of the working party on the clinical aspects and the treatment of homozygous protein C and protein S deficiencies. They will continue their work for the report at the next meeting. Then, S. Gandrille (France) introduced the database of mutations of protein S deficiency, comprising 126 entries. Lastly, T. Koide (Japan) introduced a newly identified vitamin K-dependent protein with anticoagulant property and high species-specificity. Second part of the meeting was Serpins and Tissue Factor Pathway Inhibitor (TFPI). D.A. Lane (UK) announced the completion of antithrombin mutation database: 2nd (1996) update which is being submitted as an official SSC Communication in *Thrombosis & Haemostasis*. He also proposed that the future update of the database will be made on Internet, and this proposal was accepted at

the meeting. Next, F.C. Church (USA) talked about a comparison of three heparin-binding serpins: antithrombin, heparin cofactor II and protein C inhibitor. He showed the common part and different part of the mechanism in the interaction of heparin and three well-known heparin-dependent plasma coagulation inhibitors. The last two papers were made on TFPI. J.-B. Hansen (Norway) talked on the role of lipoprotein-associated TFPI, and showed that LDL-bound TFPI lack anticoagulant function due to carboxy terminal truncation and that the anticoagulant function of TFPI is restricted to its free form in plasma. Lastly, P. M. Sandset (Norway) made the final report on the interlaboratory study on the assay of TFPI, and at the end of his talk he asked the audience if there was a need for another study and it was approved. The meeting ended 30 min. behind the scheduled time as usual.

5. One proposal of an organization of the second international working group on Standardization of Antithrombin Concentrate was made by Dr. Elaine Gray (National Inst for Biol. Standard & Control, Fax +44-707-646730).

Platelet Immunology

Chair: Cecile Kaplan, France

Co-Chairs: D. Beardsley, USA; James Bussel, USA;
Tom Kunicki, USA; Sanford Shattil, USA

Minutes were prepared by J. Bussel and C. Kaplan. Three topics were discussed: alloimmunity, autoimmunity and heparin-induced thrombo-cytopenia

Alloimmunity:

News from the European Symposium on Platelet Immunology (Helsinki): C. Kaplan

The platelet nomenclature is still a matter of concern and is the responsibility of the International Platelet Workshop of the ISBT-ASH. For two antigens, Groa and Oea, the molecular polymorphisms have been described. It still appears that GPIIIa is highly polymorphic and immunogenic. The genetic background of alloimmunisation due to anti-HPA-5b has been described and clearly differs from the one linked with anti-HPA-1a. HPA-5b immunization is associated with a cluster of HLA DR molecules sharing Glu-Asp at position 69-70 of DRb1 chain and TAP2 dimorphism Ile-Ile at position 379, whereas in HPA-1a, a major role has been attributed to DRB3, DQB1 genes and TAP1. Therefore for definition of high risk groups regarding platelet alloimmunisation, not only the platelet antigen must be considered but also the genetic background which can be different for each antigen.

Fetal Alloimmune Thrombocytopenia and therapy: J. Bussel, C. Kaplan

J. Bussel presented the results of a prospective study which has just been published in the American Journal of Obstetrics and Gynecology in May 1996. The purpose of this study was to compare IvIgG alone to IvIgG and corticosteroids as maternal therapy to reverse fetal thrombocytopenia and avoid intracranial hemorrhage (ICH). In this study 55 women were included. No ICH occurred during this therapy. Low doses of corticosteroids don't seem to add any beneficial effect. But high dose steroids had an additive effect to that of IvIgG. The overall results were good, with success of therapy in 62% - 85% of cases. This was in contrast with the results obtained by the European group who observed failure of therapy in 30% of cases with sometimes ICH, and probably no effect of therapy in 30% of cases. These apparent discrepancies could be in part explained by the means of evaluation which is very complex: not all affected fetuses will bleed, and the only marker is the evolution of the platelet counts. At the moment there is no predictive parameter for therapy effectiveness.

The natural history of this affliction is as follows: Fetal thrombocytopenia appears very early during pregnancy and is severe. The next fetus is usually more severely affected. There is no prediction for the first fetal platelet count from the sibling history. When serial platelet counts are performed during pregnancy without therapy, there is a decrease in platelet count. There is no spontaneous increase in platelet counts.

Non-radioactive PCR-SSCP for identification of new alloantigen polymorphisms : O. Peyruchaud

O. Peyruchaud gave a very interesting application of this technique allowing the characterization of yet another new antigen (Laa) located on GPIIIa nearby HPA-1. This shows once more the immunogenic property of GPIIIa.

Autoimmunity

PAICA, a new sensitive technique to characterize autoantibodies : A. Nurden

This technique relies on immobilization of platelet lysates by mouse monoclonal antibodies (MoAbs) coated on microtiter plates. Due to the specificity of the MoAbs, the target of the autoantibodies could be easily identified. This technique seems to be more sensitive than the MAIPA, perhaps because it includes total platelet IgG, not just surface IgG. It has also been used for assessing the platelet bound 7E3 in patients undergoing anti-thrombotic therapy.

Anti CD36 antibodies: N. Tandon

These antibodies have been found in TTP and also in ITP, HIV-ITP and HUS patients. Fewer plasmas from HUS, ITP and HIV-ITP patients induced significant secretion of serotonin as compared to TTP. The mechanism seems to be different but the clinical significance is not established.

Heparin-induced thrombocytopenia (HIT)

Laboratory testing: J. Amiral

In HIT, some patients are only positive with the Elisa test (H-PF4), and some only with aggregometry. In the Elisa positive, aggregation negative patients, it appears that these are largely IgA mediated. In 15% of cases, in which antibodies against H-PF4 are absent although there is HIT and the aggregometry test is positive, antibodies against IL-8 and NAP-2 neutrophil chemokines have been found. These antibodies are not heparin dependent.

FcγRII receptor and HIT: C. Bachelot

On platelets only FcγRII is found and is of low affinity. The role of His/Arg 131 polymorphism in human platelet activation by monoclonal antibodies (MoAbs) has been studied. The binding of the Fc domain of IgG1 MoAbs is higher on Arg/Arg than IgG2a MoAbs. The binding of MoAbs on FcγRII was greater on platelets from homozygous Arg donors than on platelets from homozygous His donors. Aggregation is correlated with that binding. Clinical assessment of patient populations with HIT and controls show that Arg/Arg individuals were less sensitive to HIT (only 9% were found in the HIT group). These results are similar but not identical to previously published work (Brandt et. al) and may have clinical implications. The FcγRII polymorphism should be considered when antibody-dependent platelet activation or destruction is involved.

Thrombin generation in HIT: Implications for treatment: T. E. Warkentin

HIT induces disturbances in procoagulant/anticoagulant balance. There is a high thrombin generation in HIT and venous limb gangrene could be observed. HIT-IgG is a potent generation of platelet microparticles with procoagulant activity which explains the increase in thrombin generation, and strong association with venous thrombosis and syndrome of limb gangrene. Warfarin may aggravate this tendency by decreasing protein C levels without immediately having an effect on thrombin. This gives a rationale for use of agents that suppress thrombin generation in HIT (e.g., Danaparoid, Hirudin). Warfarin should be resumed until the platelets have normalized and the patient is clinically improved.

Hirudin treatment for HIT: A. Greinacher

A prospective study was performed with 80 patients. The overall mortality was 7.3%. All fatal events were due to the severity of the underlying disease or severity of complications during therapy with heparin. Hirudin could be considered as successful in severe cases of HIT. A multinational registry was open for the natural history of HIT. Isolated HIT and the outcome was analyzed. HIT has a very high rate of thrombosis. It is necessary to determine the adverse reactions of the hirudin therapy. A multinational randomized trial comparing recombinant Hirudin (lepirudin) versus placebo in patients with HIT asymptomatic for thrombosis is proposed by A. Greinacher in Europe and T. E. Warkentin in North America. The design is a double-blind, randomized, placebo-controlled, multicenter, multinational protocol. This study will be open soon for multinational participants.

Orgaran versus Dextran 70 for HIT: B. Chong

In this randomized, controlled study the inclusion criteria was HIT and thrombosis requiring therapy. There was a stratification in two groups; stable or minor thrombosis or severe thrombosis. The overall response to therapy was 86% for Orgaran + Warfarin vs 53% for Dextran + Warfarin for complete and partial recovery. The overall clinical response was 92% for Orgaran vs 50% for Dextran. No serious side effects were reported in either treatments.

Assessment procedure for post-marketing drug adverse reaction during Heparin therapy: Ph. Nguyen

It is a legal duty to report side effects of therapy. HIT is the most severe side effect of heparinotherapy, but a definite diagnosis of HIT is difficult to establish. The proposed procedure is an objective, well-established method to evaluate the possible casual relationship between a drug and a given clinical or extra clinical event. The intrinsic imputability relies on chronological and semiological criteria. This pharmacovigilance approach can be used to identify HIT. However, to improve the quality of the information, it will be necessary to adapt each criterion to the specific case of HIT, and to evaluate the available diagnostic tests. The method should allow an estimate of the true incidence of HIT. It should be used in clinical trials, when heparin is co-administered with a new drug, in acute situations (thrombolysis, platelet inhibition, post-angioplasty).

Platelet Physiology

Chair: John L. McGregor, France

Co-Chairs: E. Angles-Cano, France; K. J. Clemetson, Switzerland

The platelet physiology subcommittee meeting in Barcelona was divided into two parts which ran respectively for one hour and forty-five minutes (part I) and two hours and 30 minutes (part II). The number of people attending this subcommittee meeting was estimated as between 70 to 80.

Part I: Platelet Physiology in Fibrinolysis (Co-Chair: E. Angles-Cano, France). The first speaker (Lindsey Miles, USA) reviewed new developments and frontiers on plasminogen binding and activation on platelets. The presence and the assembly of a number of molecules present on platelets and implicated in fibrinolysis was discussed. A new atherogenic and thrombogenic connection, between lipoprotein (a) and platelets was then reviewed by the second speaker (Eduardo Angles-Cano, France). The speaker showed the role of LP(a) in coronary heart disease. LP(a) was shown to bind to fibrin, extracellular matrix proteins and to a number of cells such as endothelial cells, mononuclear cells and activated platelets. Nuala Booth (UK) talked about platelets as fibrinolytic inhibitors and the role of PAI-1. Platelets were shown to contain small amounts of a-antiplasmin and PAI-1 is the major inhibitor of fibrinolysis in platelets.

Part II: New Platelet Inhibitors and Inhibitor Targets (K.J. Clemetson, Switzerland). Giovanni de Gaetano (Italy) reviewed the benefits and problems of new platelet inhibitors. The clinical efficacy of present drugs suggests either the search for new drugs and/or the use of available drugs in combination (for e.g. aspirin + ticlopedine). The next speaker (Graham Jamieson, USA) clearly showed the presence of a platelet high-affinity thrombin receptor, in addition to the cloned moderate affinity thrombin receptor, on the platelet surface. He presented new data on the platelet high-affinity thrombin receptor through the use of blocking monoclonal antibody and proteolysis studies. Graham Jamieson made the following suggestions to the physiology subcommittee: (1) Use highly purified a-thrombin (greater than 3000 U/mg) of stated specific activity; (2) Use of physiological relevant range of a-thrombin concentration (0.2U/ml); (3) Avoid the self-fulfilling prophecy. The third speaker (Lina Badimon, Spain) showed the platelet response to different types of ruptured plaque. She then presented the use of different platelet inhibitors, using blood that was not anticoagulated in contact with human vessel walls bearing atherogenic plaques, in a flow chamber at different shear rates. The last speaker (Mike Barnes, Cambridge) showed the presence of a new collagen receptor, not yet identified, that plays an important role in platelet activation by collagen. He used a series of synthetic peptides, derived from collagen, to indicate the presence of this new collagen receptor.

The audience indicated its approval for the introduction of a platelet bleeding disorder patient directory. Such a directory will be initiated with the help of scientists on both sides of the Atlantic. Dr. Debbie French from Prof. B. Coller's laboratory (Mt. Sinai, Hospital, NY) has already indicated her willingness to set up such a registry for Glanzmann's thrombasthnia patients.

Homepage address (<http://laennec2.univ-lyon1.fr/SCIENTIFIQUE/PLATELET/physiology.html>). This will provide a forum to discuss platelet physiology topics and to prepare future meetings. It will also contain a registry of genetic platelet bleeding disorders and schedule and reports of present and past meeting. All suggestions are welcome (mailbox: platelet@laennec.univ-lyon1.fr).

Predictive Values of Hemostatic Variables in Vascular Disease

Chair: Gordon D. O. Lowe, UK

Co-Chair: Y. Stirling, UK; K. Bauer, USA; M. Hultin, USA; R. Hull, Canada

Dr. Lowe had been asked to continue as acting chairman during 1996, and had asked Dr. Stirling to continue as co-chairman for this meeting in the absence of other co-chairmen.

Overview of Predictive Value of Hemostatic Variables in Vascular Disease: Dr. Lowe reported that this was almost complete and would be circulated soon within the subcommittee prior to submission to SSC. Fibrinogen: Dr. Lowe reported results from the Scottish Heart Health Study confirming the predictive value of fibrinogen (Clauss assay) for CHD events in men and women, especially fatal events. Using the international standard, the upper tertile (in which the relative risks was about 2.0) was defined as about 3.0 g/L. Dr. Lowe reported that in the inter-laboratory comparison of Clauss fibrinogen, published variation appeared largely attributable to variations in standards. Dr. S. Kitchen reported continuing wide variation between "routine" laboratories for "normal" fibrinogen assays in UK-NEQAS, although this may be decreasing with increasing use of the International Standard.

Factor VII: Dr. Stirling reported the results of a 20-centre calibration exercise for the SSC standard. A potency of 1.02 units/ampoule by factor VIIc assays was accepted. Data for chromogenic, ELISA and factor VIIa assays was encouraging but insufficient for formal analyses. A report would be prepared for publication. Dr. Kitchen reported good agreement between laboratories for factor VIIc assays in UK-NEQAS. Dr. Stirling suggested preparation of guidelines on factor VIIc assays in epidemiological studies and assay of the SSC standard by the Northwick Park assay. Dr. Kluff suggested comparison of factor VII assays in "high risk" samples.

Factor VIII/von Willebrand Factor: Dr. I. Jennings reported wide variation between laboratories for factor VIIIc and vWF antigen assays in UK-NEQAS. Variation in reference plasmas appeared most important, but variation in substrates and deficient plasmas also had an effect.

Fibrinolysis: Dr. C. Kluff reported the current standardization program of the Fibrinolysis Subcommittee, which was an integrated approach to standards and methods.

Coagulation Inhibitors: Dr. I. Walker reported the design and preliminary results of the EPCOT Study, a prospective case-control study of thrombotic risk in persons with congenital deficiencies in coagulation inhibitors. Drs. F. Rodeghiero and G. Lowe reported distributions and associations of antithrombin, protein C, protein S and heparin cofactor II in two large population studies. Reference ranges could not be defined parametrically, and varied with age, sex, and hormonal, lipid and lifestyle factors. Dr. I. Jennings reported wide variation for antithrombin, protein C, protein S, and APC resistance between laboratories for both assays and diagnosis in UK-NEQAS. Assay methods, source of kits, reference plasmas, expression of results and definition of reference range were each contributory.

Gene Polymorphisms: Dr. L. Iacovello reviewed gene polymorphisms related to haemostatic factors and risk of arterial vascular disease. An ETRO Working Group has been established to study the relationships to geographic variation in CHD risk across Europe. Dr. Iacovello proposed a register of such studies. Dr. N. Sala proposed a new nomenclature for existing and future gene polymorphisms.

von Willebrand Factor

Chair: J. Evan Sadler, USA

Co-Chairs: Paul Foster, USA; David Ginsburg, USA;
Dominique Meyer, France; Francesco Rodeghiero, Italy

The Subcommittee met Sunday, June 23, at the Fira Palace Hotel in Barcelona, Spain, from 8:00 to 12:00.

Presiding chair was Dr. Dominique Meyer. Dr. Sadler and Dr. Ginsburg did not attend. Other co-chairs were present.

Attendance was approximately 80.

Recent developments in VWD were presented in a session chaired by Dr. Dominique Meyer. Dr. Claudine Mazurier summarized the discovery of new mutations in VWD during the past year. Dr. Meyer reported on the use of the PFA-100TM (Dade International) for screening in VWD, and on an automated latex bead immunoassay for VWF (Diagnostica Stago). Dr. Emmanuel Favaloro described the use of a collagen binding assay (VWF:CBA) in the laboratory assessment of VWD.

During the past year, the Subcommittee has developed consensus criteria for the diagnosis of VWD type 1. Dr. Pier Mannucci chaired a session in which these criteria were presented and discussed. Dr. Francesco Rodeghiero summarized the criteria for the use of patient history and family history. Dr. Giancarlo Castaman summarized the consensus approach to laboratory tests. Dr. Mannucci presented the specific criteria for the diagnosis of VWD and possible VWD. Several suggestions for modification of the criteria were offered. By a vote of the members these criteria were APPROVED for further evaluation in patient populations after consideration of the suggested modification by the working party.

Dr. Paul Foster chaired a session to consider Subcommittee projects for the next year. Dr. Rodeghiero proposed a retrospective study of consensus criteria for VWD type 1. A proposal for the retrospective validation of the diagnostic criteria in two patient populations was made and the creation of a Working Party on VWD Diagnosis for this purpose was proposed and members recruited. Dr. Foster discussed issues of standardization for patient populations and laboratory tests. Creation of a Working Party on VWF Assays was also proposed and members recruited. This Working Party will address the standardization and evaluation of laboratory tests in VWD, including VWF:CBA and other candidate assays in the diagnosis of VWD.

The following article will be published during 1996:

Mazurier C, Meyer D: Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease. Results of an international survey. *Thromb Haemostas*, in press.