Meetings

Hemophilias

The three most common types of inherited bleeding disorders are hemophilia A (classical hemophilia), hemophilia B (PTC deficiency, Christmas disease), and vascular hemophilia (von Willebrand's disease). Hemophilia A is due to the lack of a plasma coagulation factor known as factor VIII (antihemophilic factor AHF), while hemophilia B results from a deficiency of factor IX (plasma thromboplastin component, PTC, Christmas factor). Vascular hemophilia is characterized by low levels of factor VIII and a long bleeding time.

In severe hemophilia A and severe hemophilia B, excessive hemorrhage may occur in any part of the body. Hemarthroses and subcutaneous and intramuscular hematomata frequently develop. Vascular hemophilia is in general a milder disorder, and bleeding is characteristically from mucous membranes and, in women, from the endometrium, although severe hemorrhage in other areas may occasionally occur. Patients afflicted with these bleeding disorders live in constant fear of exsanguination. Frequent hospitalization and repeated plasma transfusions are the rule and result in heavy financial and social burdens for the hemophiliac and his family.

The pressing problems of patients with hemophilia have stimulated intensive investigation of the biochemical and physiological nature of factors VIII and IX. The ultimate aim of this research has been to develop purified and concentrated preparations of these factors for fundamental investigation and for clinical use. Because of the many recent advances and the numerous problems in these areas, the current status of basic and clinical research on factors VIII and IX was recently discussed at the 3rd International Conference on Hemophilia in Washington, D.C. (7–8 December 1963). Particular attention was paid to the fundamental roles played by factors VIII and IX in the generation of thrombin, and to the purification of these factors. Advances in the clinical management of patients with hemophilia A and hemophilia B were also discussed.

Currently there are several methods for fractionating factor VIII from human plasma. One readily available human fraction is the Merck Sharp & Dohme fibrinogen product (obtained by the Cohn cold ethanol fractionation procedure), which contains 5 to 7 times the factor VIII activity of a similar amount of human plasma in terms of protein concentration. The Blombäcks reported on their factor VIII preparations, which contain from 10 to 50 times the specific factor VIII activity of the starting plasma. This preparation, fraction I-O, is the residue from a glycine extract of Cohn fraction I. By the addition of tannic acid to fraction I-O, contaminating fibrinogen is precipitated, a technique which has permitted Pavlovsky and Casillas to make human factor VIII products 200 times purified. Wagner and Webster have developed a simple procedure in which beta-alanine is employed as the precipitant, and which yields a human factor VIII fraction 60 times purified with respect to the starting plasma.

Factor VIII fractions made from animal plasma were also discussed. Bidwell presented data on commercially available factor VIII concentrates from porcine and bovine plasmas which are calculated to be about 300 times purified, on the basis of protein concentration. These fractions have been used extensively in the treatment of patients who require high concentrations of factor VIII for hemostasis. Wagner reported that canine factor VIII fractions, precipitated with aliphatic amino acids, have a specific activity up to 2000 times that in plasma.

As yet, factor IX fractions comparable to the best factor VIII concentrates have not been obtained. Bidwell has been able to prepare factor IX concentrates by tricalcium phosphate adsorption of the G-2 fraction of Kekwick and Mackay. These factor IX fractions were reported to be useful in the treatment of hemophilia B. Hink and Johnson described their purification procedure, which is based on the adsorption of factor IX to barium sulfate and its subsequent elution in 4 percent sodium citrate. Mammen and Seegers reported that they have isolated and purified autoprothrombin II from purified prothrombin. They believe that autoprothrombin II is identical with factor IX and that it is a derivative of the prothrombin molecule.

The difficulty in the purification of factors VIII and IX is compounded by their small concentrations in the starting plasma. In addition, the instability of factor VIII is particularly troublesome, especially during manipulations necessitated by purification procedures.

For diagnostic purposes both factor VIII and IX can be adequately measured by one-stage techniques based on the partial thromboplastin time of Langdell, or by two-stage techniques such as the thromboplastin generation test of Biggs and Douglas. These two tests are similar in reproducibility and accuracy, although Ingram believes that the partial thromboplastin time test, with added activation product, has definite advantages over the thromboplastin generation test. Both tests are affected by multiple and sometimes uncontrollable variables that lead to results which may be difficult to interpret. This is particularly true when these assays are used to test purified products or to detect the so-called hypercoagulable states.

Another problem with the current assays is that there is no common standard for either factor VIII or factor IX. This situation prompted Aronson to quote Proverbs 20:10, "Divers weights, and divers measures, both of them are alike abomination of the Lord." Until a common standard can be established for both factor VIII and factor IX it will be difficult to relate the work of one laboratory to that of another. In addition, the lack of reference standards will make it difficult to accurately label purified products of either factor VIII or IX.

Generally, factor VIII activity in plasma varies from about 50 to 200 percent of the activity in a normal control. Egeberg showed that increased

levels of this factor are present in diabetes, coronary atherosclerosis, hyperthyroidism, malignant diseases, and pregnancy, whereas lowered levels occur in hypothyroidism. Experimentally, levels of factor VIII have been increased by exercise or by the administration of adrenalin, pyrogens, blood and milk, and oral contraceptive drugs. The increased levels of factor VIII in these and other conditions have been interpreted as representing a hypercoagulable state consistent with a tendency to develop thromboembolism. Penick pointed out, however, that these changes in factor VIII levels may be an artifact of the nonspecificity of assays for factor VIII. He also emphasized that as yet there has been no definite correlation of increased levels of factor VIII and thromboembolic diseases.

There is some evidence that factor VIII is activated during the clotting process, but this has been difficult to establish because this factor is consumed during coagulation. In vitro experiments by both Penick and Rapaport showed that very small amounts of thrombin are capable of activating factor VIII, as judged by shortening of clotting times. This may at least partially explain why the recorded values for factor VIII concentrations are sometimes supernormal.

Perhaps one of the most significant controversies relating to factor VIII today is whether there is actually a deficiency of this factor in the plasma of patients with classical hemophilia or whether its presence in these patients is masked by an inhibitor. Mammen presented evidence that hemophilic plasma inhibits factor VIII activity when purified prothrombin is used as the assay substrate. This view is supported by the finding that factor VIII-like activity can be recovered from hemophilic plasma on extraction with ether. It has been shown, however, that infusion of such plasma into hemophilic dogs does not have a correcting effect on prothrombin consumption.

The precise role of factor IX in the blood-clotting mechanism is not known, although it is thought to be important in the first phase of coagulation. There is an increasing amount of evidence to suggest that factor IX exists in plasma in precursor form and is possibly changed to the active form by factor XI (plasma thromboplastin antecedent). Aggeler reported that factor IX activity in fresh serum is 3 to 6 times that in plasma. The high factor IX activity of serum may be more apparent than real, since infusion of serum into patients with hemophilia B does not result in the expected increase in the plasma concentration of factor IX.

Aggeler and Loeliger both showed that the biological half-life of factor IX in patients with hemophilia B is 24 to 28 hours. This is in contrast to the 12- to 24-hour half-life generally ascribed to factor VIII. According to Adelson, the half-life of factors VIII and IX, as determined in normal subjects by means of a radioisotope tagging method, is expressed in days rather than hours. Why the half-life of these two factors should be different in normal and in hemophilic subjects is not known, but methodological differences may account in part for this large discrepancy.

Whereas vascular hemophilia is inherited as an autosomal dominant characteristic, classical hemophilia is inherited as a sex-linked recessive disorder. This suggests that factor VIII synthesis is regulated by genetic loci on at least two chromosomes. This is supported by the findings of Swedish and French investigators who reported that in vivo, but not in vitro, "complementation" occurs when blood from a patient with classical hemophilia is infused into patients with vascular hemophilia. Graham presented data to show that the factor VIII so "synthesized" in vascular hemophiliacs cannot be differentiated from normal factor VIII on the basis of *p*H and heat stability. This led him to speculate that the mutation in von Willebrand's disease is of a regulator gene, while the mutation in classical hemophilia is of a structural gene. A functional relationship of the low factor VIII level to the long bleeding time in vascular hemophilia has not been shown. The Blombäcks described a plasma fraction which was devoid of factor VIII activity but was capable of shortening the bleeding time. They concluded that the long bleeding time in vascular hemophilia is due to the absence of the so-called "vascular factor."

Many of the advances in basic research related to factors VIII and IX have been directly applicable to the clinical management of patients. The judicious and intensive use of plasma in the therapy of these disorders has revolutionized the management of all types of hemophilia. Rosenthal pointed out that hemophilia is a stereotyped clinical syndrome which lends itself to "programmed" management which can greatly reduce the complications of these disorders, particularly the chronic crippling which so often results from hemarthroses.

Plasma alone, however, is insufficient for the treatment of many severe bleeding episodes. Biggs pointed out that in these instances there is an obvious need for potent purified factor VIII and IX fractions. Both human and animal factor-VIII products have been used with success both in the treatment of serious bleeding and prophylactically for major surgery.

The conference members stated that improved medical care may well diminish the current sociological problems faced by the hemophiliac, since the decrease in days lost because of sickness improves educational and vocational opportunities, and these in turn diminish the heavy financial burdens of the hemophiliac and his family. The question was raised of whether the increase in longevity of hemophiliacs might not increase the incidence of the hemophilia gene in the population. Li pointed out that this was unlikely and that proper genetic counseling to patients and parents should offset any slight increase which might occur.

The conference ended by recommending that there be an increase in training programs for physicians interested in the various aspects of hemophilia; that additional centers for the comprehensive care of hemophiliacs be established in institutions with clinical and research interests in the hemophilia problem; and that sufficient amounts of blood, plasma, and plasma fractions be made available for treatment of hemophiliacs.

The conference proceedings are being published by the University of North Carolina Press; K. M. Brinkhous is editor. This will be the third proceedings volume of this series; the earlier ones were published in 1957 and 1959. The conference, held under the auspices of the Medical Advisory Council of the National Hemophilia Foundation, was supported by grants from the Gustavus and Louise Pfeiffer Research Foundation and from the National Heart Institute (HE-08-479-01).

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