C. K. Kasper, L. Aledort, D. Aronson, R. Counts, J. R. Edson, J. v. Eys, J. Fratantoni, D. Green, J. Hampton, M. Hilgartner, P. Levine, J. Lazerson, C. McMillan, J. Penner, S. Shapiro and N. R. Shulman (U.S.A.): **A More Uniform Measurement of Factor VIII Inhibitors. (339)**

A group of hematologists, involved with hemophilia research and care in the U.S.A., met under the sponsorship of the Division of Blood Diseases and Resources of the National Heart and Lung Institute. In order to improve future communication among ourselves, we decided to alter our individual methods of measurement of inhibitors to the extent necessary to permit a uniform, although arbitrary, description of inhibitor units. We agreed to the following standards: (1) The incubation mixture consists of one part citrated patient plasma, undiluted or diluted, plus an equal part of citrated pooled normal human plasma. (2) A control incubation mixture consists of equal parts of normal pooled plasma and imidazole buffer, as formulated by Dr. Biggs. (3) The mixtures are incubated at 37° C for two hours. (4) Assays specific for Factor VIII are then performed and the Factor VIII activity in the patient mixture is divided by the Factor VIII activity in the control mixture to determine the percent residual Factor VIII activity. (5) A patient plasma giving a residual Factor VIII activity of 50 percent in this test is said to contain one "Bethesda unit" of inhibitor per ml. (6) On a graph, the log percent residual Factor VIII activity is plotted against inhibitor units. If the residual Factor VIII activity of the incubation mixture is between 75 and 25 percent, the inhibitor units are read from the graph. Plasmas containing strong inhibitors are diluted with imidazole buffer before being placed in the incubation mixture. A dilution is sought which will result in a residual Factor VIII activity between 75 and 25 percent. The units of inhibitor read from the graph are then multiplied by the dilution factor to determine the number of Bethesda units of inhibitor per ml of undiluted patient plasma.

We invite interested colleagues to join us in the use of this method, and we invite discussion of better methods of describing inhibitor potency.

J. W. Hampton, A. Weidenbach, G. E. Skye, C. Rubenstein and F. B. Taylor (Oklahoma Medical Research Foundation, 825 N.E. 13th, Oklahoma City, Oklahoma, U.S.A.): **Hemophilia: Modified by a Post-Exercise Plasminogen Activator. (340)**

A family with 9 members of both sexes in 4 generations was demonstrated to have enhanced fibrinolysis associated with excessive bleeding. Three with the worst bleeding, all males, had 10% Factor VIII activity (high antigen, bleeding time normal and ristocetin platelet aggregation normal) with no male to male transmission. Antiplasmin proteins, α-1 antitrypsin and α-2 macroglobulin were present and functionally normal. Inconsistently elevated fibrin degradation products, low fibrinogen and short euglobulin lysis times first suggested that a plasminogen activator might be present and enhanced by physical activity. Epsilon amino caproic acid (4 gms/day) given to one member with low Factor VIII inhibited the plasma activator but his Factor VIII activity only rose from 10 to 30%. In 2 affected members tested after exercise the activator activity increased up to 10-fold and required exogenous plasminogen for its demonstration. Ammonium sulfate precipitation (35–45%) of the post-exercise plasma was pH, temperature and concentration dependent and again required exogenous plasminogen. At optimal conditions for plasminogen activation plasmin was obtained with the ammonium sulfate precipitation. Chromatography of the precipitate on Sephadex G-200 showed separation of the activator from both plasminogen and fibrinogen. The protein showed no reaction with an anti-urokinase on double-immunodiffusion and did not require Hageman fragments for its activation. We conclude that the Factor VIII deficiency in this family more closely resembles classic hemophilia and that exercise-induced activator excess modifies the expression of the genetic disorder. The presence of the activator is not Factor VIII dependent since the addition of Factor VIII *in vitro* did not inhibit the activation of plasminogen.