COMPARATIVE STUDY OF TWO PROCEDURES FOR DETERMINATION OF FACTOR VIIIR:AG LEVELS IN NORMAL AND HEMOPHILIC POPULATIONS. L.E. Strike, B.Saint-Paul and J.P. Allain. French Red Cross, La Queue-les-Yvelines and C.T.S., Versailles, FRANCE.

Factor VIIIR:AG levels were determined in three populations: 37 normal males (NM), 36 hemophiliacs (H) and 36 hemophiliacs with antibodies to factor VIII (HA). The procedures used were Laurell electroimmunodiffusion (EID) and radioimmunoassay (RIA) according to Hoyer. The same rabbit antibody was used for both technics. The overall correlation between the two procedures was r=0.72. When divided according to populations, the correlations were: NM r=0.90, H r=0.72, and HA r=0.60. The clinical state of the patients was then considered, i.e., the presence of bleeding or evidence of liver disease as compared to the quiescent state. In the H population using EID, the mean VIIIR:AG level in 23 quiescent patients was 1.18±0.45 u/ml and was significantly higher in 13 non-quiescent patients, 1.52±0.28 u/ml. Using RIA, VIIIR:AG levels were 1.10± 0.37 u/ml and 1.24±0.25 u/ml respectively. The correlation between the two technics was relatively high (r=0.77) for the H quiescent group and low (r=0.43) for the H-non-quiescent group. In the HA group VIIIR:AG levels in 15 quiescent patients were 1.27±0.41 u/ml with EID and 0.91±0.29 u/ml with RIA. The correlation between the technics was r=0.71. These results indicate that to properly measure VIIIR:AG levels attention should be given to the clinical state of the patient at the time of sampling, and that different technics may measure different molecular forms of the factor VIII molecule.

AN INTERNATIONAL COLLABORATIVE STUDY OF FACTOR VIII. T.W. Barrowcliffe and T.B.L. Kirkwood National Institute for Biological Standards and Control, London, U.K.

A collaborative assay was organised to assess the suitability of a replacement for the first International Standard for Factor VIII. Coded samples of a freeze-dried concentrate (proposed 2nd I.S.), the 1st I.S., and a freeze-dried plasma were assayed by 15 laboratories against fresh normal plasma and local standards. Ten laboratories performed 1-stage assays and five 2-stage.

The proposed 2nd I.S. had a mean potency of 1.14 International Units per ampoule by direct assay against the 1st I.S., with no significant difference between one- and two-stage assays. When assayed against a large number of individual normal plasmas, the proposed standard was equivalent to 1.05 ml "average normal plasma" per ampoule. In assays of the common freeze-dried plasma against the 1st I.S., there was a significant difference between assay methods, the 1-stage assays giving higher results for the plasma than the 2-stage. This difference between assay methods confirms results from other collaborative studies, and it seems likely that the 2-stage method is detecting relatively more activity in the concentrate standards.

It was agreed by the participants that the proposed material is suitable, in terms of stability and comparability with other materials, to serve as the 2nd International Standard for Factor VIII. The standard was established by WHO at the 28th meeting of the Expert Committee on Biological Standards, with a potency of 1.1 IU per ampoule.

ALTERATION OF COAGULATION IN INTENSIVELY TRANSFUSED HEMOPHILIC PATIENTS. Helen S. Hathaway, Roger D. Hamstra, Linda Jacobson, and William E. Hathaway, University of Colorado School of Medicine, Denver, Colorado, U.S.A.

Bleeding may occasionally occur in adequately transfused hemophilic patients. To investigate this phenomenon, 11 patients with classical hemophilia had serial coagulation studies performed during intensive transfusion therapy with factor VIII concentrates given for surgical procedures. The studies included kaolin partial thromboplastin times (KPTT), fibrinogen, monomer, and fibrin split products (FSP) levels, and assays for factor VIII by the one-stage PTT method (PTT-VIII), thromboplastin generation time method (TGT-VIII), and immunologic method (VIII Ag). After an initial correcting dose, factor VIII concentrates were administered once to twice daily in a dose to keep the minimal level above 20 percent. Alterations of coagulation assays were most pronounced 7-10 days postoperatively. These included (1) KPTT values at least 20 seconds longer than expected for the percent factor VIII; (2) TGT-VIII levels consistently higher than PTT-VIII levels (mean difference was 20 percent); (3) VIII Ag values from 216-660 percent of normal. Fibrin monomer and FSP tests were frequently positive and fibrinogen levels ranged from 300-700 mgm percent. Four patients exhibited spontaneous wound bleeding on postoperative days 7, 7, 9, and 12 in spite of adequate factor VIII levels. These studies and the results of in vitro experiments with factor VIII concentrates suggest that altered proteins or degradation products of fibrinogen or factor VIII may produce spurious values for coagulation tests and may be associated with an increased bleeding tendency and/or abnormal wound healing.