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0561 THE WFH/ICTH STUDY OF THE SENSITIVITY OF DIFFERENT PARTIAL THROMBOPLASTIN TIME TESTS IN MILD HAEMOPHILIA

P.F. O'Brien, W.R.S. North and G.I.C. Ingram*, Department of Haematology, St. Thomas' Hospital, London; and Epidemiology and Medical Care Unit, Northwick Park Hospital, Harrow, Middlesex, U.K.

The second phase of the WFH/ICTH study has been conducted by distributing some 40 snap-frozen coded plasma samples from normal subjects and mild haemophiliacs to seven U.K. laboratories, where they were tested with the Manchester PTT reagent (Dr. L. Poller) and seven commercial reagents. Each laboratory also assayed each sample (under different code) for VIIIc by the local method. Each laboratory used the Manchester reagent and a given pair of the commercial reagents throughout the study, so that each commercial reagent was used by two different laboratories. The correlation between VIIIc and PTT was imperfect, with consistant discrepancies across reagents for given samples, making clear that the PTT was affected by other plasma characteristics (presumably within the normal range) besides the low VIIIc. The ability of reagents to discriminate between ca 30iu/dl and 100iu/dl VIIIc appeared to be correlated with the slope of PTT on VIIIc in the range 5-30 iu/dl. The results from mild haemophiliacs and normal subjects will be reported in detail for each PTT reagent.

P6-109 0562 PHOTOMETRIC ASSAY OF FACTOR VIIIC WITH A CHROMOGENIC SUBSTRATE

H. Vinazzer Hemophilia Center Linz,Austria

By the aid of chromogenic substrates,highly specific assays of some serine proteases can be carried out. The substrate used for factor VIIIC-assays was Bz-Ile-Glu-Gly-Arg-pNA (S-2222 KABI) which measures factor Xa. When all components necessary for factor Xa activation except factor VIIIC are kept at constant levels,the resulting Xa-activity is in direct relation to the concentration of factor VIIIC. The substrate plasma was a mixture of hemophilia A plasma with factor VIII inhibitor plasma with a remaining inhibitor activity of between 0.1 and 0.5 units per ml. This substrate was defibrinated by ancrod. For assays of factor VIIIC,this plasma was mixed with the diluted test plasma,cephaloplastin and calcium chloride at 37°C. After a constant activation time,the chromogenic substrate was added and the difference in OD/min was measured at 405 nm. The calibration curve was linear between 1% and over 200% factor VIIIC activity,and the average CV was 7.9%. This method was compared to a standard one-stage method for factor VIIIC. Identical results were obtained in plasma samples of normal individuals,in samples of high factor VIIIC activity,in plasma from hemophilia A patients,and in factor VIII concentrates. The advantages of this method over the clotting method are: independence of the results from variations of factors V,II,and I in the reaction mixture,stability of the reagents,a better discrimination of factor VIIIC levels in the range between 30% and over 100%,and the possibility of automatization of the method.

P6-110 0563 A TENTATIVE CAUSE OF DISCREPANCY BETWEEN FVIII:C ESTIMATION.

M.J. Seghatchian. North London Blood Transfusion, Edgware. Middx. U.K.

Biochemical analysis based on the molecular size and change was performed on normal, patient plasmas and clinical concentrate to differentiate various molecular forms of FVIII:C. Assay of the effluent fractions by one stage, two stage and chromogenic methods revealed two major peaks of activities, measured to differing extents by various methods. Thromboplastin generation methods (two stage clotting and chromogenic) were particularly sensitive to smaller forms, which had a faster electrophoresis mobility and eluted in the gamma globulin region. The one stage method measured mainly the aggregated forms (MW >300,000), with the slower electrophoretic mobility.

A discrepancy of about 30% between the two methods could account for the detection of the smaller form, which predominated in plasma rather than concentrate. This had a preferential adsorption on Al(OH)3 and some FVIII:C neutralizing activity. A similar peak of activity was identified in haemophilia and V.Wd. plasma isolated on sepharose suggesting that the whole plasma contained an inhibitory factor acting on the clotting activity. Excess factor X and traces of thrombin-like enzymes could be identified in this region. Thus the role of these factors in accelerating the rate of FXa generation by activating FVIII can not be excluded.

Regardless of the molecular nature of this form of FVIII, this activity may play an important role in controlling haemstasis via VIII activation and may explain the discrepancy between assay of plasma versus concentrate.