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FACTOR VIII IS A CALCIUM-BINDING PROTEIN. W.H. Cruickshank, E.S. Tackaberry, D.S. Palmer and G.A. Rock*. Canadian Red Cross Blood Transfusion Service, Ottawa Centre, Ottawa, Ontario, and *University of Ottawa, School of Medicine, Ottawa, Ontario.

The high molecular weight complex of Factor VIII is known to dissociate into two subunits when chromatographed in a high ionic strength solution containing either calcium or sodium salts. Calcium also has an essential role in maintaining Factor VIII activity during storage (AABB 1980). These two findings suggest an integral involvement of calcium in the basic structure-function relationship of the Factor VIII molecule. Consequently, we have examined the binding of ^{45}Ca to both the low molecular weight (LMW) procoagulant subunit of Factor VIII and the high molecular weight (HMW) complex using PAGE and column chromatography on Sepharose 4B. When the HMW complex of Factor VIII was isolated from cryoprecipitate by standard chromatographic procedures, incubated with $^{45}CaCl_2$ and subjected to polyacrylamide gel electrophoresis, the resultant autoradiogram demonstrated that all of the ^{45}Ca was associated with the HMW material which did not enter the gel. When this HMW Factor VIII was incubated with ^{45}Ca and then dissociated by column chromatography in a buffer containing 0.25 M $CaCl_2$, all of the ^{45}Ca was associated with the material eluting in the 2.3 V_o region. This corresponds to the elution volume of the LMW, procoagulant subunit of Factor VIII. Dialysis against buffer was not effective in removing the ^{45}Ca. The data indicate that calcium is tightly associated with the low molecular weight subunit of Factor VIII and presents further corroborative evidence that calcium has an essential role in determining both the chemical and physical properties of the Factor VIII molecule.

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STABILIZATION OF FVIII:C BY CALCIUM. G.A. Rock*, W.H. Cruickshank, E.S. Tackaberry and D.S. Palmer. Ottawa Centre of the Canadian Red Cross Blood Transfusion Service, Ottawa, Ontario and *University of Ottawa, School of Medicine, Ottawa, Ontario.

The well-known two phase decay curve of Factor VIII which has been attributed to enzyme degradation is not altered by the addition of specific protease inhibitors. However, complete stability of Factor VIII, for at least 24 hours, is accomplished when physiological levels of calcium are maintained. Addition of calcium plus heparin to whole blood at 0, 2, and even 4 hours, effectively restores Factor VIII activity to 0 time values and maintains this activity throughout the next 24 hours. The restoration of activity noted after 2 and 4 hours.of incubation is very rapid with the Factor VIII activity increasing within 15 minutes of addition of calcium. Data obtained from column chromatography indicates that the molecular distribution of Factor VIII between high and low molecular weight forms is affected both by time and by the level of calcium present in blood. When blood is collected either into heparin or a mixture of CPD plus heparin plus calcium chloride, the elution profile indicates that Factor VIII activity is equally distributed between high and low molecular weight forms, both at 0 and 24 hours with the total activity remaining unchanged during this time interval. However, when Factor VIII activity at 0 time is in the high molecular weight form with less than 10% in the low molecular weight form. By 24 hours there is virtually no low molecular weight activity remaining in this citrated plasma. The data demonstrate an absolute requirement for calcium in the maintenance of Factor VIII:C activity and suggests that the relative lability of Factor VIII during the first phase of the decay curve is due to the loss of VIII:C activity when calcium is not present at physiological concentrations to stabilize the molecule.

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AN APPARENT EFFECT OF SIZE DISTRIBUTION OF FVIIIR:AG MULTIMERS ON IMMUNORADIOMETRIC ASSAYS. M.A. Lamb, H.M. Reisner, H.A. Cooper, R.H. Wagner. Dept. of Pathology and Center for Thrombosis and Hemostasis, University of North Carolina, Chapel Hill, N.C., USA.

Immunoradiometric assays (IRMA) of FVIIIR:Ag from normal and certain variant VWD plasmas have suggested possible antigenic differences in the molecules. Studies reported thus far have used antibody specific for normal FVIIIR:Ag. We have further studied this question of antigenic differences using 2 populations of antibody isolated from an antisera prepared against highly purified human FVIII. Isolated IgG was labeled with [125-I]. The population of labeled IgG "specific" for variant FVIIIR:Ag was separated by immune complex formation with a VWD plasma previously shown, by 2% agarose crossed immunoelectrophoresis, to contain only the lower molecular weight multimers of FVIIIR:Ag. The "specific" labeled IgG was obtained by low pH dissociation and subsequent G-200 chromatography. [125-I] IgG "specific" for normal FVIIIR:Ag was similarly obtained after immune complex formation with pooled normal human plasma. Liquid phase IRMAs were performed using differential precipitation with ammonium sulfate or PEG to separate antigen-antibody complexes from free antibody. Using antibody "specific" for normal FVIIIR:Ag, a lack of parallelism was noted in the dose-response curves of variant plasmas as well as a decrease in maximum antibody bound, as compared to normal. Interestingly, when this antibody was absorbed with the variant VWD plasma and the remaining antibody used in IRMAs, none was bound by either variant or normal plasma.

Using antibody "specific" for variant FVIIIR:Ag, a similar lack of parallelism in dose-response curves and a decrease in maximum antibody bound were observed. Therefore rather than antigenic differences as previously implied, these results suggest that the discrepancies noted in IRMAs of variant and normal plasmas are a function of the size of the FVIIIR:Ag multimers.

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A METHODOLOGICAL INVESTIGATION ON THE ONE-STAGE FACTOR VIII ASSAY. J. Over, J.A. van Mourik, P. van den Brink-Zantingh and R. Smit-Jansen. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Assay of Factor VIII coagulant activity (VIII:C) in Factor VIII concentrates has since long met difficulties, such as 1) non-parallellity of dose-response curves of plasma standard and Factor VIII concentrate, 2) spuriously low values of VIII:C in concentrates as revealed by abnormally high in vivo recoveries after transfusion, and 3) large interlaboratory variation in assay results. In an attempt to analyze the cause of these problems several parameters of the one-stage assay system were varied systematically and their effect on the parallellity of dose-response curves and on the final VIII:C value was analyzed. Nonparallellity was partially corrected with a protein-rich dilution medium, and almost always completely with undiluted instead of 1:1 diluted hemophilic substrate plasma. In both conditions apparently higher VIII:C values were found.

A number of assay systems used by different producers of Factor VIII concentrates were compared. The standard and, in some cases, the phospholipid reagent seemed to contribute for the largest past to the interlaboratory variation, but also other, as yet unidentified, factors exerted some influence. These findings initiated a cooperative study by five Red Cross Blood Transfusion Services in Europe on standardization of the one-stage assay for VIII:C. This resulted in a better correspondence between these institutes (CV 13%) compared to the previous situation (CV 23%).

It is concluded that 1) substrate plasma should not be diluted, especially when Factor VIII concentrate is to be tested against a plasma standard, 2) the standard should be of the same type as the testmaterial, and 3) this standard should be properly calibrated against the International Standard for Factor VIII.