

THE PASSOVOY DEFECT: FURTHER CASE REPORTS AND PRELIMINARY CHARACTERIZATION. C. Hougie, J. E. Brown, P. Lakin-Thomas, R. A. McPherson, L. Aronson and R. F. Baugh. Department of Pathology, University of California, San Diego, School of Medicine, La Jolla, California, U.S.A.

Since the first report of a patient with a deficiency of the Passovoy factor (Lancet II:220, 1975) we have encountered 10 other cases from six kindred. The patients have a small but significant prolongation of the activated partial thromboplastin time which is corrected by normal plasma. They have normal levels of all the hitherto reported clotting factors, including Fletcher and Fitzgerald, no evidence of an inhibitor, and no mutual correction. The condition is transmitted as an autosomal dominant. Most of the patients have had a moderate to mild bleeding diathesis, thus the proband in the original kindred recently bled profusely into the neck three days following the removal of a small cervical node and was given 6 units of blood to control the bleeding. Attempts to characterize the factor have been hampered by the relative insensitivity of the assay method; however a fraction has been found which shortens the clotting time of Passovoy deficient plasma, but not plasmas deficient in other clotting factors; the biochemical properties of this factor are currently under investigation.

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PHARMACOKINETICS OF HUMAN FACTOR IX IN A DOG WITH SEVERE HEMOPHILIA B. P.A. Gentry, A.R. Thompson and A.W. Forrey. Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada and Hematology, University of Washington and Puget Sound Blood Center, Seattle, WA, U.S.A.

In preparing a factor IX concentrate with a high yield and low hepatitis and thromboembolic risks, we have tested this material for survival in an *in vivo* system, the hemophilic dog. By following the disappearance of radiolabeled, isolated factor IX in addition to the classic clotting assays, data on protein survival and more accurate kinetic parameters were obtained.

Crude factor IX concentrate was prepared by batchwise adsorption-elution with DEAE-Sephadex using cryoprecipitate-poor human plasma. Isolated human factor IX was radiolabeled with  $^{125}\text{I}$  by chloramine-T without *in vitro* loss of clotting activity (Thompson, *J Clin Invest*, in press, 1977). A preparation containing both crude and isolated factor IX was then subjected to filtration (0.22  $\mu\text{m}$ ) and lyophilization; clotting and radioactivity were not altered by these steps.

Following infusion of the combined preparation into a dog with severe hemophilia B (0% baseline factor IX) 10 post infusion samples were taken over 96 h for determination of radioactivity and factor IX clotting activity. These data were then analyzed by fitting to a two exponential expression using a Marquart non-linear least squares numerical procedure for a two compartment open model. The central volume was 14.5% of the animal's body weight; the total volume of distribution was 28% with a  $t_{1/2}$  distribution of 114 min. The  $t_{1/2}$  elimination was 20 h; the slower phase of elimination ( $\beta$ , or that affected by redistribution) had a  $t_{1/2}$  of 40 h. Factor IX clotting activity from the crude concentrate closely paralleled radioactivity from the isolated factor IX throughout the 96 h;  $t_{1/2}$   $\beta$  was slightly longer from the clotting activity data.

IN VITRO THROMBOGENICITY TEST FOR CLINICAL FACTOR IX CONCENTRATES USING SYNTHETIC SUBSTRATES M. Miller-Andersson, AB Kabi, Stockholm, Sweden. M.J. Seghatchian, North London Blood transfusion Centre, Edgware, U.K.

Venous thromboses have been reported following the clinical use of some factor IX preparations. This may be due to several causes as for instance traces of  $\text{FX}_a$  or thrombin formed during processing or presence of surface activation products involved in the early stages of the coagulation pathway. There is an urgent need for simple and reliable thrombogenicity tests. Activation by  $\text{Ca}^{2+}$  should preferentially be avoided, as  $\text{Ca}^{2+}$  by itself is capable of activating the prothrombin complex. All clotting methods are affected by the heparin content (4-5 u/ml) and excess amount of sodium citrate present in the F IX concentrates. We have therefore developed a colourimetric method using synthetic substrates. The assay is performed in the absence of presence of known proteolytic inhibitors in order to improve the specificity of the test system and identify the nature of the thrombogenicity. Using this method, various concentrates can be classified in three groups according to the rate of cleavage of the substrates. Hot materials have been titrated with several inhibitors. The contaminating enzymes have also been isolated and characterized. The results clearly indicate the presence of two entirely different types of hot material. One group contains mainly  $\text{FX}_a$  and thrombin and can be neutralized by AT III. The other group probably contains large amounts of surface products. The "thrombogenicity" of this group can be mimicked by kaolin activation of plasma and inhibited by Trasylol.