

1083 THE EFFECT OF AUTOPROTHROMBIN II-A ON THE DIC FORMED ANIMALS

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Autoprothrombin II-A was isolated from bovine prothrombin. DIC was induced in experimental animals with thromboplastin obtained from the brains of rabbits. An hour after thromboplastin administration, which had been injected very gradually from the ears of a group of rabbits, the comparative coagulation tests with previously taken blood were performed. In the second group, inhibitor along with thromboplastin was injected to the ear and the same tests were repeated comperatively with blood taken previously. The autoprothrombin II anticoagulant had protective effect on DIC formation with rabbit brain thromboplastin administration. This protective effect was found to be statistically significant.

1084 CHANGES IN PLATELET FUNCTION AND PLATELET COUNTS DURING SUBSTITUTION THERAPY IN HAEMOPHILIACS AND PRODUCED BY PLASMAPHERESIS IN PATIENTS SUFFERING FROM INHIBITORS

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We noticed a systematic increase in small platelets (evaluated by electronical analysis of platelet volume distribution, using the Coulter Counter equipment, Wenzel 1977) during substitution therapy in patients suffering from haemophilia (N = 60). Laboratory investigations on these patients were performed before substitution and then 30 min., 60 min., 120 min. and 24 hours after infusion of factor-VIII-concentrations (Immuno, Schwab, Behring, factor-VIII-concentrates 20 U/kg b.w.). The same investigations were performed before and after plasmapheresis using a Hemonetric cell separator (N = 7). In 48 of the patients, the clinical signs were insignificant (bleeding time, according to Duke, was found to be normal), although the platelet changes were considerable (decrease in platelet count and increase of the percentage of platelets smaller than $4.5 \mu^3$). However, significant test results were noticed in a haemophiliac patient suffering from inhibitor- and drug-induced platelet disorders during and after plasmapheresis. We observed bleeding complications only in 2 cases (Duke: 7 min. and 9 min.). Yet, a considerable decrease in platelet counts was observed as well as a significant increase in the percentages of small platelets ($4.5 \mu^3$, N = 48) in all cases. Controlling platelet function in haemophiliacs following substitution therapy could be essential as well as controlling the usual hemolysis parameters after plasmapheresis.

1085 FRACTIONATION AND RECONSTITUTION OF THE VITAMIN K DEPENDENT PROTHROMBIN CARBOXYLATION SYSTEM

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A specific enzyme which uses vitamin K hydroquinone plus O_2 and CO_2 for the carboxylation of either endogenous protein or synthetic peptide has been difficult to isolate. The activity appears to be lost during isolation. Two different methods have been found whereby partial fractionation of the system and reconstitution will give recovery of active carboxylation. *In vivo* injection of vitamin K eliminates endogenous substrate. Triton solubilized microsomes from these animals show essentially no carboxylation of endogenous protein. Addition of purified prothrombin precursor to this system leads to carboxylation both of this endogenous substrate and also of pentapeptide acceptor. These data indicate at least two proteins needed for enzyme function. Soluble system can be fractionated by removal of Triton by XAD, and passing the pellet dissolved in minimal Triton through phenyl sepharose to yield 3 inactive fractions (the XAD supernatant, the void volume from the phenyl sepharose column and a Triton eluate). While each fraction is individually inactive, recombination of void volume and eluate fraction or of XAD supernatant and eluate restore carboxylation of both endogenous protein precursor and of added pentapeptide.