0363

β-PROPIOLACTONE/ULTRAVIOLET IRRADIATION: QUANTITATIVE STUDIES ON EFFECTIVENESS FOR INACTIVATION OF HEPATITIS B VIRUS. A.M. Prince[1,3,4], W. Stephan[2], and B. Brotman[1,3]. [1]The Lindsley F. Kimball Research Institute of The New York Blood Center, New York, New York, [2]Biotest-Serum-Institut GmbH, Frankfurt, Germany, [3]The Liberian Institute for Biomedical Research, Robertsfield, Liberia, [4]Dept. of Pathology, New York Hospital-Cornell Medical Center, New York, New York.

The efficacy of combined β-Propiolactone/Ultraviolet irradiation (βPL/UV) for inactivation of hepatitis B virus in labile blood derivitives has been reinvestigated. To permit quantitative estimation of process efficacy, a regression analysis of the relation between virus dose and incubation period was carried out. This has provided a means for estimating virus titer and for determining the accuracy of such estimates.

The data suggest that βPL/UV can reduce virus titer about 10,000,000 fold (10^{-7}). The process efficacy for βPL/UV followed by the Aerosil adsorption procedure used in preparation of stabilized human serum (Biseko®) containing most human serum proteins except for removal of factor VIII, the PPSB factors, fibrinogen and the lipoproteins was estimated to be about 10^{-8}.

This degree of virus inactivation should be more than sufficient to sterilize the highest amounts of hepatitis B virus which could be expected in pooled human plasma which has been screened for HBsAg.

0364

VITAMIN K DEPENDENT CARBOXYLATION OF PEPTIDE BOUND GLUTAMIC ACID RESIDUES. D.P. Kosow, M.P. Esnouf, A.I. Gainey, H.A.O. Hill and P.J. Thornally. Department of Clinical Biochemistry and Inorganic Chemistry Laboratory, University of Oxford, U.K.

The chemical mechanism by which vitamin K promotes the posttranslational carboxylation of specific glutamic acid residues in the N-terminal region of prothrombin has not yet been elucidated. We have previously suggested that vitamin K reacts with dioxygen and carbon dioxide to form a species of active carbon. In this study we have investigated the reaction of reduced vitamin K in alcoholic solution with dioxygen in the presence and absence of carbon dioxide. We find that carbon dioxide is necessary for the rapid formation of vitamin K oxide. Vitamin K oxide was formed when either *cis* or *trans* vitamin K was used. However, *trans* vitamin K is specifically required in enzymatic carboxylation studies. We propose that in rat liver microsomal preparations the carboxylation of synthetic peptide substrates is coupled to the chemical epoxidation of vitamin K by the carboxylase.

0365

ISOLATION AND CHARACTERIZATION OF THE VITAMIN K DEPENDENT DOMAIN OF HUMAN PROTHROMBIN. C. Dodé, A. Thiesce, D. Labie, and J. Elion. INSERM U.15, Institut de Pathologie Moléculaire 24, rue du Faubourg Saint-Jacques, 75014 Paris, France.

Chymotryptic cleavage of human prothrombin ($_h$Pt) produces two fragments of 68000 and 5000 MW respectively. These two species were separated by Ba citrate adsorption and Sephadex G100 chromatography. The 68000 MW species corresponds to $_h$Pt (des 1-44) and has lost all the vitamin K dependent properties of $_h$Pt : adsorption on Ba citrate, Ca^{+2} and phospholipid dependent stimulation of the activation by FXa, presence of strong Ca^{+2} binding sites (as shown by dialysis equilibrium) and Ca^{+2} induced fluorescence quenshing. The 5000 MW species corresponds to the N-terminal portion of $_h$Pt (residues 1-41). It contains the 10 Gla residues and the 17-22 disulfide bond. This peptide is quantitatively adsorbed on Ba citrate. Activation of $_h$Pt in a mixture containing FXa, Ca^{+2} and phospholipid is drastically inhibited by the addition of peptide 1-41 (≃ 50 % inhibition for a 1/1 peptide/Pt ratio, ≃ 7 % for a 40/1 ratio).

Ca^{+2} produces a quenshing of the intrinsic fluorescence of peptide 1-41 (λ emission = 355 nm). This quenshing plateausat 40 % of the initial fluorescence at 3 mM Ca^{+2}. The plot of the fluorescence quenshing versus Ca^{+2} concentration however is not cooperative. Mn^{+2} and Mg^{+2} also induced fluorescence quenshing but to a lesser extent. Hence peptide 1-44 represents a functionnal domain in itself interacting with Ca^{+2} and phospholipid. It contains only one Trp (residue 41), showing directly the involvment of this residue in the Ca^{+2} induced fluorescence quenshing observed for Pt fragment (F1) and Pt. This isolated vitamin K dependent domain therefore retains many of the vitamin K dependent properties of F1 or Pt, but shows differences in the Ca^{+2} induced conformational change in that it is no longer a cooperative process.

0366

PROTHROMBIN FRAGMENT 1•2•3, A MAJOR PRODUCT OF PROTHROMBIN ACTIVATION IN HUMAN PLASMA. M.J. Rabiet, B. Furie, and B.C. Furie. Tufts-NEMCH and Tufts Univ. Sch. Med., Boston, MA.

The conversion of human prothrombin to thrombin is associated with a number of cleavage intermediates and products whose appearance and concentration are dependent upon the prothrombin activation conditions used. In the current investigation, the fragments of prothrombin which appear in normal human plasma after activation of the blood coagulation cascade were studied. Radioiodinated human prothrombin was added to platelet-poor relipidated normal human plasma and clotting initiated with Ca(II) and kaolin. The radiolabeled prothrombin cleavage products which formed were analyzed by polyacrylamide gel electrophoresis in the presence of dodecyl sulfate (SDS) and 2-mercaptoethanol (2-ME). A new product of prothrombin activation was observed. Its migration was more rapid than prethrombin 1 and slower than fragment 1•2. No previously identified products of prothrombin activation migrated to the same position in the gel.

The previously unrecognized fragment was identified as fragment 1•2•3 as follows. Prothrombin was activated by factor Xa in the presence of Ca(II) and phospholipid. The desired product was isolated by absorption to and elution from barium citrate and by DEAE cellulose chromatography. This purified material, migrating identically with the unknown plasma product was homogeneous upon SDS gel electrophoresis with 2-ME. The amino terminal sequence of the isolated material was identical to that of prothrombin. Digestion of this material with either factor Xa or thrombin yielded as major products fragment 1•2 and fragment 1. (Fragment 2 and fragment 3 eluted from the gels under the conditions employed). Amino terminal sequence analysis of the factor Xa digestion products of the isolated material indicated three amino acid residues at each cycle. The sequences of fragment 1, fragment 2, and fragment 3 are consistent with this sequence analysis. On this basis we suggest that fragment 1•2•3 is a prominent product of prothrombin conversion to thrombin in plasma.