THE CONVERSION OF BOVINE ABNORMAL PROTHROMBIN INTO PROTHROMBIN. C. Vermeer, B.A.M. Soute, H.C. Hemker. Department of Biochemistry, State University Limburg, Maastricht, The Netherlands.

In order to characterize the enzyme system which converts abnormal prothrombin (PIVKA-II) in prothrombin we prepared liver cell-free extracts from normal healthy cows. The enzyme system devoid of either precursor or prothrombin was isolated by differential centrifugation and partly purified with the aid of column chromatography. The carboxylation of purified PIVKA-II to prothrombin was detected by means of the one-stage coagulation assay and with $^{14}\text{CO}_2$ incorporation. The reaction was dependent upon microsomal supernatant, vitamin K, ATP, Mg^{2+} ions, O_2 , pH and temperature. Inhibitors were: warfarin, 2,4-dinitrophenol and factor II. From our experiments we concluded that PIVKA-II can act as a precursor of prothrombin and that PIVKA-II can be converted into the normal clotting factor in an in vitro cell-free system. In contrast to the systems published by others, here the converting enzymes are added to separately purified precursor proteins, so the system provides a way to study the nature of this reaction in more detail.

CHARACTERIZATION OF MULTIPLE FORMS OF DICOUMAROL-INDUCED PROTHROMBINS AND THEIR FRAGMENTS, F1. O.P. Malhotra, VA Hospital and Case Western Reserve University, Cleveland, Ohio, U.S.A.

To confirm that multiple forms of prothrombin are induced by dicoumarol, we isolated prothrombins from the plasma of dicoumarol-treated steers and divided them into three groups: 1) barium (Ba) citrate variant adsorbable by Ba citrate; 2) Ba oxalate material adsorbable by Ba oxalate but not by Ba citrate; and 3) the alumina atypical proteins(s) adsorbable by alumina C γ -gel but not by the Ba salts. The purified atypical varieties showed a single component by several physicochemical criteria and contained as much antigenic material as did normal prothrombins, but bound different amounts of Ca^{2+} . The Ca^{2+} -binding ability of these proteins depends on the carboxylation of the glutamyl residues on the amino portion of prothrombin. The extra carboxyl groups appeared to have little influence on the isoelectric points of prothrombins because the Ba citrate variant binding Ca^{2+} , equal to 38% of the normal, focused at the same pH (pH 4.55 or 4.6) as normal prothrombin. Ba oxalate proteins bound Ca^{2+} equal to 25% of normal and focused at pH 4.75; the alumina variant(s) bound little, if any Ca^{2+} and focused at pH 4.82. To prove unequivocally whether or not multiple forms of dicoumarol-induced prothrombins are produced, normal and atypical prothrombins were digested separately with thrombin to yield Fragment 1 (F1) and Intermediate 1 (I1). Both F1 and I1 were obtained in highly purified forms by employing DEAE-cellulose chromatography, gel-filtration, and affinity chromatography on heparin-agarose. Since the Ca^{2+} binding sites reside in F1, they were characterized (Ca $^{2+}$ -binding abilities, electrophoretic mobilities, antigenic properties and gel-isoelectrofocusing). In general, these experiments confirmed our studies with intact proteins: i.e., that different forms of atypical prothrombins are induced by dicoumarol and they differ from each other primarily in the extent of carboxylation of the glutamyl residues present in the amino portion of prothrombin.

PARTICIPATION OF γ -CARBOXYGLUTAMIC ACID RESIDUES IN METAL BINDING: ^{13}C NMR STUDIES OF THE INTERACTION OF LANTHANIDE IONS WITH BOVINE PROTHROMBIN FRAGMENT (12-44). Furie, B.C., Blumenstein, M., and Furie, B. Tufts Univ. School of Medicine, Boston, MA, U.S.A.

Comparative studies of Ca(II) binding to bovine prothrombin and abnormal prothrombin have led to the hypothesis that γ -carboxyglutamic acid (Gla) residues either participate directly in metal liganding or stabilize the tertiary structure of the protein to facilitate metal binding, or both. Extending previous studies of the binding of lanthanide ions to factor X, prothrombin and prothrombin fragment 1, the interaction of lanthanide ions with prothrombin fragment (12-44), which contains eight Gla residues, has been examined by natural abundance ^{13}C NMR spectroscopy at 67.88 MHz. The relationship between bound metal ions and carbon atoms in fragment (12-44) was determined using paramagnetic Pr(III) and Eu(III) based upon the strategy that the magnitude of the change in the chemical shift of resonances of the carbon atoms induced by bound metals is related to the interatomic distance between bound metal and carbon nuclei. Assignments of the resonances in the ^{13}C spectrum of fragment (12-44) were based upon our studies of the chemical shifts of carbon resonances in DL-Gla and known chemical shifts of carbon resonances of the other amino acid residues. Titration of fragment (12-44) with Pr(III) or Eu(III) was accompanied by a downfield or upfield shift, respectively, of the γ -carboxyl carbon resonances and no shifts were observed for other carbon resonances. The shift of resonances within the γ -carboxyl carbon envelope appeared selective, suggesting the non-equivalence of the relationship of the γ -carboxyl group of the eight Gla residues to the bound metal ions. These data offer direct evidence that some, but perhaps not all, Gla residues participate in metal liganding.