HUMAN PROTEIN C, ITS INHIBITOR, AND THE COMBINED DEFICIENCY OF FACTORS V AND VIII. <u>Richard A</u>, <u>Marlar and John H</u>. <u>Griffin</u>, Scripps Clinic and Research Foundation, La Jolla, California 92037 USA

Human protein C (PC) is a vitamin K-dependent serine protease zymogen present in normal plasma. PC was activated with soluble thrombin, thrombin-Sepharose, trypsin-Sepharose or Russell's viper venom. When activated protein C (APC) at 10  $\mu$ g/ml was added to normal plasma for 3 min prior to assays, clotting was greatly inhibited. In prothrombin time, activated partial thromboplastin time, and partial thromboplastin time assays, the clotting times were prolonged from 24 to 68 sec, 57 to 616 sec, and 247 to > 1200 sec, respectively. Both calcium and phospholipid were needed for this potent anticoagulant activity. The thrombin time was unaffected. To test which coagulation factors in plasma were inactivated by APC, APC was added to normal human plasma for 3 min and residual clotting factor activities were assayed using appropriate deficient substrate plasmas. Only Factor V and Factor VIII activities were not anticoagulant and did not decrease Factors V and VIII. APC hydrolyzed synthetic peptide substrates. The amidolytic activity of APC was inhibited by normal human plasma (50% of APC activity was lost in 10 min). APC inhibitory activity was not detectable in plasmas from 4 unrelated patients with combined Factor V/VIII deficiency whereas it was present in normal amounts in plasmas from patients with simple Factor V and YIII. Moreover, APC tiself may be regulated by a previously undescribed plasma protein inhibitor. It is suggested that the molecular basis for combined Factor V/VIII deficiency of APC inhibitor.

## 0182

REGULATION OF BOVINE ACTIVATED PROTEIN C BY PROTEIN S: THE ROLE OF THE COFACTOR PROTEIN IN SPECIES SPECIFICITY. <u>F.J.</u> <u>Walker.</u> Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, IN, USA.

The anticoagulant activity of activated Protein C has been observed to be species specific. This could be due either to the inability of the bovine enzyme to recognize its substrate, Factor Va, in non-bovine plasmas, or the absence of cofactor-Protein S, a protein that has been shown to be necessary for the maximum expression of the anticoagulant activity of activated Protein C. Activated Protein C was found to be an effective inhibitor of Factor Xa-initiated clotting of bovine plasma, but without acti-vity in either human or rabbit plasma. Human and rabbit plasma supplemented with bovine Protein S was sensitive to the anticoagulant activity of activated Protein C. Neither rabbit nor human plasma contained bovine activated Protein C cofactor activity as measured by the enhancement of bovine activated Protein C-catalyzed inactivation of Factor However, bovine activated Protein C was able to in-Va. activate both human and rabbit Factor Va. The inactivation of both of these proteins could be stimulated by the addition of bovine Protein S. These results indicate that the species specificity of bovine activated Protein C is due to the absence of a cofactor protein in non-bovine plasma that will interact with the bovine enzyme. Secondly, these findings further confirm that Protein S is required for the maximal expression of the anticoagulant activity of activated Protein C.