

FREE COMMUNICATIONS IV

Coagulation: Contact Activation.

MOLECULAR PROPERTIES OF BOVINE HAGEMAN FACTOR. H. Claeys, B. Van Hoef and D. Collen. Lab. of Blood Coagulation, Dept. of Medical Research, Univ. of Leuven, Belgium.

Hageman factor was isolated from bovine plasma by CM-Sephadex, Ultrogel AcA-44 and DEAE-Sephadex chromatography, with a yield of 1.1 ± 0.2 mg per liter plasma and a specific activity of 51 ± 12 units per mg protein. The amino acid composition was similar to that reported for Human Hageman factor (Revak et al. 1974). The preparation previously characterized in our laboratory (Vth Cong. Paris 1975, Abstract n° 118) now appears to have been contaminated with a histidine rich protein. The present material could be adsorbed to and eluted from celite without loss of activity and without apparent generation of spontaneous activity.

By SDS-polyacrylamide gel electrophoresis, two molecular forms of bovine Hageman factor could be distinguished: a single chain molecule with an estimated molecular weight of 80,000 and a two chain disulfide bonded molecule with mol.wts of 50,000 and 32,000. The amount of the two chain protein in different preparations varied between 0.25 and 1.00.

Incubation with DF³²P revealed that the two chain protein molecule was completely inhibited with the incorporation of 1.2 mol of DFP per mol of protein, whereas the single chain molecule did not incorporate DFP. These data suggest that the single chain molecule is a precursor form with inaccessible active site serine and the molecule in which the two chains are connected by disulfide bridge(s) is an activated or degraded form with accessible active serine. Similar structural properties have recently been described for human Hageman factor, in which the two chains of the activated form are however not disulfide linked (Griffin et al. 1976).

ACTIVATION OF HAGEMAN FACTOR (HF, FACTOR XII) BY SEPHADEX-ELLAGIC ACID MIXTURES. O. D. Ratnoff and H. Saito. Case Western Reserve University School of Medicine, Cleveland, Ohio, U.S.A.

In the intrinsic pathway of clotting, PTA (Factor XI) is activated by activated HF. Whether activation of HF requires the presence of prekallikrein (Fletcher factor) and high M.W. (HMW) kininogen (Fitzgerald factor), or whether these agents act after activation of HF is disputed. Purified HF activates PTA in the absence of prekallikrein, but this may reflect molecular changes in HF during purification.

Sephadex G10 or G15 was mixed with ellagic acid and freed of excess ellagic acid by washing. A mixture of normal plasma, or plasma deficient in HF, prekallikrein, HMW kininogen or PTA, and Sephadex-ellagic acid was centrifuged at 31000 xg. Each supernatant plasma except that deficient in HF shortened the abnormally long partial thromboplastin time (PTT) of HF-deficient plasma. Plasma simultaneously depleted of HMW kininogen, prekallikrein and PTA also shortened the PTT of HF-deficient plasma and of plasma depleted of HF and prekallikrein (albeit minimal clotting times were reached more slowly in the latter case) but had virtually no procoagulant effect upon the PTT of plasma depleted of HF and HMW kininogen.

Thus, exposure of HF in plasma to Sephadex-ellagic acid generated a clot-promoting form of HF in the absence of other clotting factors, but its full expression required the presence of HMW kininogen.