

ASYNCHRONOUS GENERATION OF KININ AND KALLIKREIN ACTIVITY FOLLOWING SOLUBLE ACTIVATION OF PLASMA. A.M. Bubnic, T.R. Zuffi and M.A. Fournel. Research Division, Cutter Laboratories, Inc.

In an effort to assess the physiological significance of a soluble activator of Factor XII, the kinetics of F. XII and prekallikrein activation and subsequent kinin formation have been investigated using dextran sulfate. Employing an amidolytic assay for kallikrein and a bioassay for kinin activity a reproducible lag in the generation of detectable kallikrein activity has been demonstrated in the presence of explosive kinin generation. Time, temperature and concentration kinetics have been determined as well as characterization of this system with prekallikrein activator (PKA; B-XII<sub>a</sub>; XII<sub>f</sub>), selective inhibitors and deficient plasmas. These studies have shown that kinin activity is generated in substantial amounts prior to the generation of any significant kallikrein activity by the activation of F. XII, suggesting that the physiological role of prekallikrein activation in kinin generation may be relatively minor. *In vivo* animal studies with PKA appear to confirm this finding with insignificant alterations in prekallikrein titers in the presence of marked systemic kinin-mediated hypotension.

EVALUATION OF THE PLASMA KALLIKREIN SYSTEM IN ARGENTINE HAEMORRHAGIC FEVER (FHA). E. Giavedoni, F.C. Molinas and J.I. Maiztegui. Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Buenos Aires and Centro de Estudio de Fiebre hemorrágica argentina, Pergamino, Argentina.

Previous work in this laboratory have demonstrated that the coagulation and complement systems are involved in Argentine haemorrhagic fever (AHF). Due to their interrelation with the plasma kallikrein system we have decided to evaluate the latter measuring Factor XII (F XII), prekallikrein (PK) and kallikrein inhibitors (KIs) in 18 patients with AHF. The patients were grouped according to clinical parameters in the following forms: mild (15 patients), moderate (2 patients) and severe (1 patient). Values of F XII, determined with F XII deficient plasma, were within normal range. The concentrations of PK and KIs (enzymatic assay) in AHF patients were respectively: days 5-7, 106.9 uM/ml/h, 0.99 U; days 8-10, 97.4 uM/ml/h, 0.98 U; and days 11-12, 104.8 uM/ml/h, 0.92 U. These levels were similar to our normal controls, however the 2 patients with the moderate clinical form presented on days 9-11 values lower than normals (PK 58.6 uM/ml/h; KIs 0.82 U). Simultaneously we investigated the lengthening of the partial thromboplastin time activated with kaolin (PTTK) observed in these patients. In order to demonstrate the presence of an early phase inhibitor we determined the PTTK using mixtures of patient and normal plasmas incubated for 20 min at 37°C. The results obtained discarded the presence of such an inhibitor. Our study indicate that there is no activation of the plasma kallikrein system in this group of patients. Further studies are needed with more cases of the moderate and severe clinical forms of AHF.

THE FACTOR XII-INDEPENDENT PLASMINOGEN PROACTIVATOR SYSTEM OF PLASMA INCLUDES UROKINASE-RELATED ACTIVITY. C. Kluff, G. Wijngaards and A.F.H. Jie. Gaubius Institute TNO, Leiden, The Netherlands.

Antibodies to urokinase have been shown to quench part of the fibrinolytic activity of plasma, not involving the extrinsic system. Previously, the intrinsic system has been subdivided into two parts, one dependent and another independent of factor XII, for activation. Both represent approximately 50 BAU activator activity/ml plasma. The aim of this study was to further locate the UK-related activity within the intrinsic fibrinolytic pathways and to delineate its participation in fibrinolysis tests *in vitro*.

Antibodies to UK (AUK) were shown not to quench activities of contact factors such as factor XII, prekallikrein and factor XI in coagulation and chromogenic substrate assays. AUK inhibited in normal plasma, Hageman trait and Fletcher trait plasma a discrete portion of approximately 50 BAU activator activity/ml, apparently the activator activity derived from the factor XII-independent system.

Immunoadsorption of plasma with immobilized AUK resulted in a depletion of approximately 50 BAU activator activity/ml. The contact factors were undisturbed, as were plasminogen and the extrinsic activator. Additions of UK did not restore lost activity, suggesting that AUK has removed the proactivator component.

The amount of UK-related activator activity compares to approximately 0.4 IU urokinase activity/ml, and shows a fairly constant level in individuals and is not enhanced by known stimuli for extrinsic activator release.

Significant contributions to blood fibrinolytic activity in current assay systems, including the dilute blood clot lysis time method could be demonstrated.

PRESENCE OF COMPLEMENT FACTOR C4 AND HMW KININOGEN IN PROTHROMBIN COMPLEX CONCENTRATES. M. Steinbuch, L. Pejaudier M.C. Boffa\*. Centre de Transfusion Sanguine Orsay and Paris\* XVème . France.

The protein contaminants of prothrombin complex preparations obtained by adsorption onto ion exchangers and namely DEAE-Sephadex were studied.

Among them a high amount of C4 accompanied by some C4-binding protein was noticed. Certain preparations contain as much as 20 times the normal plasma concentration of C4. This complement component is able to form an anaphylatoxin. Its molecular integrity has been checked by immunoelectrophoresis and rocked electrophoresis. Its degradation by several enzymes is shown.

The clotting component concentrates contain also high amounts of kininogen. The enrichment of high molecular weight (H.M.W.) kininogen is comparable to the one observed for C4. Its presence was assessed with an antiserum specific for this protein and the ability of the kininogen to supply kinin was checked on guinea pig ileon.

The potential thrombogenicity of prothrombin complex preparations has been attributed to contact factors. It is shown in this study that factor XII and its derivatives as well as PK can be easily removed during the processing of the fraction. However, kininogen displays the same affinity for the ion exchanger than the clotting components