**Thursday, July 16, 1981**

## Poster Presentations

# Coagulation – XVI

## Factor XII, Kallikrein, Kininogen

*11:00–12:30 h*

**Grand Ballroom Lobby Boards 217–227**

## 0721

CONTACT ACTIVATION BY ELLAGIC ACID - THE CONCEPT OF SOLUBLE ACTIVATOR DISPUTED. T. Exner and K. A. Rickard. Haematology Department, Royal Prince Alfred Hospital, Sydney, Australia.

Ellagic acid (EA) has often been described as a "soluble" activator of the contact mechanism in contrast to kaolin, silica or glass which are insoluble. We have found that EA is not effective as a contact activator when in true solution but is active only when present as a particulate solid or dispersion.

A pH titration of EA was carried out and it was found that EA dissolved readily in alkaline solutions. When adjusted to neutral pH these formed supersaturated solutions which slowly precipitated forming fine microcrystalline dispersions. At high EA concentrations there was concurrent development of turbidity and contact activity. At lower EA levels no turbidity was visible, yet contact activity increased on standing, presumably as EA particle size increased. Contact activity was reduced in all ellagic acid preparations by centrifugation after standing.

Substancesof low molecular weight in true solution do not sediment under these conditions and do not show turbidity. These are properties of particles or aggregates. These observations support the old theory that only particulate materials providing a liquid/negatively charged solid interface of substantial size function as true activators.

It is probably important to distinguish between activation of the contact mechanism by substances which may be in true solution such as various organic sulphates and those which are dispersions of solids, functioning possibly by a different mechanism.

## 0722

IDENTIFICATION OF THE PROCOAGULANT SPECIES IN ELLAGIC ACID SOLUTIONS. Paul E. Bock and Joseph D. Shore. Division of Biochemical Research, Department of Pathology, Henry Ford Hospital, Detroit, Michigan 48202 U. S. A.

The species of ellagic acid responsible for initiating intrinsic blood coagulation has been characterized. Ellagic acid is soluble at a level of 30 ± 10 μM in pH 7.4 Tris-saline buffer at 22°C. Dilution of soluble ellagic acid resulted in enhanced plasma procoagulant and kallikrein generating activity, and the appearance of a new absorbance spectrum. These effects were prevented by 1 mM EDTA, and the new species could be removed by centrifugation. Addition of stoichiometric $Cu^{2+}$ to Millipore-filtered soluble ellagic acid generated an absorbance spectrum similar to that caused by dilution in the absence of EDTA, as well as procoagulant and kallikrein activities. $Zn^{2+}$ and $Co^{2+}$ caused similar spectral changes and prekallikrein activation. Although no turbidity was visible and the spectral properties did not indicate extensive light scattering, centrifugation resulted in loss of the absorbance spectrum and activity. We conclude that the procoagulant activity of ellagic acid solutions can be ascribed to slowly settling insoluble aggregates of ellagic acid-metal ion complexes, which are formed with adventitious metal ions present in the diluting buffer. Formation of these aggregates could be prevented but not reversed by 1 mM EDTA. Although soluble ellagic acid may bind to Factor XII, it does not initiate blood coagulation or prekallikrein activation, since these activities were only associated with the insoluble species.

## 0723

UNACTIVATED PLASMA OF CHILDREN WITH COOLEY'S ANEMIA HAS SERINE PROTEASE ACTIVITY. M. Andrew, M. Manno and M. Karpatkin. Department of Pediatrics, New York University Medical School, New York, U.S.A.

Twelve children with Cooley's Anemia aged 2-17 had low biologic activity of factors XI, XII and prekallikrein (PK), (mean ± S.E., XI:56%±6; XII:51%±7; PK:59%±6). This did not appear to reflect depressed hepatic function as other factors measured (fibrinogen, II,V,VII,VIII,IX and X) were in the normal range. To investigate the possibility that these factors were being activated in vivo and then removed, nonactivated plasma collected in Na Citrate and EACA was incubated with the chromogenic substrate for thrombin, S2238. The substrate was cleaved by every patient tested (34 samples from 12 patients); nm p-nitroanaline (pNA) released/ml/min was 33±5 compared to 3±4 in 20 samples from 10 healthy controls ($p < .001$). The substrates for plasma kallikrein (S2302) and Xa (S2222) were also cleaved. S2302 showed greatest sensitivity and S2222 the least (mean nm pNA/ml/min; 2302:185±27; 2238:43±11; 2222:11±4; $p < .001$). This proteolytic activity was present in fresh plasma and remained stable at -70° for at least 1 month. It was inhibited by DFP indicating that it was a serine protease. Trasylol, which inhibits plasma kallikrein and plasmin, also inhibited it. It was unaffected by hirudin indicating that it was not thrombin. Children older than 10 years (N=6) had more protease activity than those (N=6) less than 10 (97±23 nm pNA/ml/min vs 24±6; $p < .05$). Thus patients with Cooley's Anemia have a serine protease in unactivated plasma which increases with age and may be related to iron overload. We speculate that due to increased tissue iron a zymogen (?PK) is activated to a protease (?kallikrein) which activates XII which in turn activates XI. The activated factors are then removed. This would explain the depletion of XI, XII and PK in these patients.