of calcium caused maximum release of TFa while lower or higher concentrations were less effective. Other bivalent cations (Mg, Mn, Zn) could not replace Ca ions and caused no release, while Co caused only very slight release of TFa. The release was time and temperature dependent: maximum activity being released at 37° C and almost no activity released at 4° C. When the effect of pH was studied, maximum release of TFa occured at pH 6.5–7.5. Substances which affect cell contractility such as vincristine, colchicine, cytochalasin B, as well as ouabain which affects some ATPase's had no effect on release. These data show that leukocyte tissue factor activity can be released from the cell in the presence of calcium. This release mechanism may play a role in some pathological conditions.

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P. N. Sawyer (Downstate Medical Center, Brooklyn, New York 11203 U.S.A.): **Effects of Various Fibrinolytic Agents on the Physico-Chemical Characteristics of the Cardiovascular System.** **(425)**

The effects of fibrinolytic agents Brinase, Brinolase (enzymes isolated from aspergillus oryzae) and Thrombolysin (streptokinase and human plasmin) on the cardiovascular system of dogs and rabbits have been determined by the following methods: (a) electrophoretic mobility of canine erythrocytes and platelets; (b) alterations of the vascular intimal surface charge as determined by electroosmosis and streaming potentials; (c) alterations in platelet and erythrocytes adhesion characteristics; (d) changes in coagulability of blood as demonstrated by several standard determinations; (e) histology of rabbit blood vessels, and (f) scanning electron microscopy of canine blood vessels. The results indicate that Brinase at 2 mg/kg increases the net negative surface charge of the vascular intima. There is a byphasic response in altering the electrophoretic mobility of erythrocytes and blood platelets, an initial increase in the surface negativity followed by a return to normal after two days. Brinase reduces platelet adhesion to a metal surface as well as to the vascular intima. Histologic examination of hyperlipidemic rabbit vessels indicates that Brinase is effective in reducing fat infiltration with a minimal amount of destruction to the vascular wall. Brinolase has been administered at doses 4.3 mg/kg and 1 mg/kg. The former being a full proteolytic dose results in irreversible incoagulability, total proteloysis and usually death of the animal. At 1 mg/kg the hematologic and physico-chemical effects on the cardiovascular system are similar to those produced by Brinase. The overall effects of Thrombolysin are comparable to those induced by Brinase at 2.0 mg/kg in the long term study.

P. J. Gaffney, K. Lord and R. D. Thornes (National Institute for Biological Standards and Control, London NW3 6RB and Royal College of Surgeons in Ireland, Dublin 2): **The Action of Brinase in Vitro and in Vivo.** **(426)**

Brinase (an extract of Aspergillus Oryzae) was shown to rapidly digest human fibrinogen in vitro to aggregable degradation products with a molecular size range of 310,000 to 230,000 the latter fragments being more slowly digested to core fragments, D_{br} and E_{br}. The fibrinogen polypeptide chain susceptibility to Brinase attack was in the order $A\alpha$, γ, $B\beta$. Lysis of the $B\beta$ chain seems to be the rate limiting step in the conversion of the high molecular weight fragments (MW 310,000–230,000) to the core fragments D_{br} and E_{br}. The conservation of NH_2 terminal Tyrosine during fibrinogen digestion and the very transient existence of D dimer fragments during totally crosslinked fibrin lysis suggest that the carboxy end of the γ chain is prone to Brinase attack. The crosslinked α chains of fibrin, while resistant to plasmin, are vigorously digested by Brinase. The plasma of cancer patients being treated with Brinase contained degraded fibrinogen (lacking intact $A\alpha$ chains) and their aggregates. These aggregates contained some crosslinked γ chains (γ-γ dimers) suggesting that Brinase in vivo exercises both a lytic and coagulant effect. Thrombin mediated clots in all the plasmas examined contained no crosslinked α chains. Positive plasma ethanol gelation tests can be explained by the presence of the aggregable high molecular weight fragments observed during the in vitro lysis of fibrinogen by Brinase.