A STUDY OF LYMPHOCYTE TRANSFORMATION AS A MECHANISM FOR SPONTANEOUS FACTOR VIII INHIBITOR FORMATION. J. Katz, R. Lea and E.D. Gomperts. Department of Haematology, School of Pathology of the University of the Witwatersrand and The South African Institute for Medical Research, P.O. Box 1038, Johannesburg, South Africa.

Two elderly patients with a bleeding tendency due to spontaneous Factor VIII inhibition were investigated. An *in vitro* study was designed to investigate lymphocyte transformation and the production of Factor VIII inhibitor. Blood samples from patients were separated on a ficoll-hypaque gradient and the mononuclear cells were cultured with haemophilic plasma, Factor VIII concentrate and normal plasma as sources of Factor VIII antigen. The results showed that the patients lymphocytes were stimulated by some samples of haemophilic plasma, but not by the Factor VIII concentrates or normal plasma. However, normal control lymphocytes were similarly stimulated by these samples of haemophilic plasma suggesting that immune complexes were present in the haemophilic plasma and probably caused the stimulation. Furthermore, the patients lymphocytes were stimulated with phytohaemagglutinin candida and tuberculin and normal responses were obtained with AB serum. However, the sera of both patients inhibited candida and tuberculin stimulated lymphocyte cultures and the inhibition correlated with the presence of Factor VIII inhibitor. In this study a cellular immune mechanism for the production of spontaneous Factor VIII inhibitor could not be demonstrated, however a serum inhibitor of candida and tuberculin stimulated lymphocyte cultures was found.

Inactivation of Trypsin By Antithrombin III. Richard D. Feinman and Tien-ling Chang. State University of New York Downstate Medical Center Brooklyn, N.Y., U.S.A.

We have studied the reaction of antithrombin III (AT) with trypsin as a model of the interaction of the inhibitor with the serine proteases of the coagulation scheme. From equilibrium binding studies, the reaction is reversible and binding is weaker than for the AT-thrombin reaction. Stop-flow kinetic studies show that trypsin reacts much faster than thrombin with AT; the trypsin reaction in the absence of heparin is almost as fast as the reaction of thrombin with the heparin-AT complex. Trypsin reactions, on the other hand, are relatively insensitive to heparin.

ACTIVATION OF THE COAGULATION SYSTEM BY ENDOTOXIN IN FROG (*RANA ESCULENTA* L.) G. Csákö, E. Gazdy, H. Csernyánszky, S. Tóth and T. Szilágyi. Institute of Pathophysiology, University Medical School, Debrecen, Hungary.

The frogs, unlike mammals, are extremely resistant to the lethal effect of gram-negative endotoxins. Even injecting enormous doses we were unable to determine the DL₅₀ value at their usual ambient temperature. The finding by Levin and Bang that endotoxin activates the coagulation in the invertebrate *Limulus* suggested, however, a similar effect in frogs as well. Frogs were, therefore, injected with a high dose of *E. coli* O111 endotoxin (Boivin). Four hours later the fibrinogen level was 30 %, the thrombocyte count 50 %, and the neutrophil count 70 % lower than the initial value. By 24 hours a further drop of fibrinogen level appeared, whereas the thrombocyte and neutrophil counts started to normalize. At that time, repeating the endotoxin injection (generalized Shwartzman reaction) a decrease of all 3 parameters studied was again observable in 4 hours. When frogs were treated i.p. with high endotoxin doses 72; 72 and 48; or 72, 48 and 24 hours before blood sampling, progressively prolonged prothrombin (Quick) and thrombin times were obtained, indicating a consumption coagulopathy. (Unfortunately, reptilase times are hardly measurable in frog plasma - Blut 30:283,1975) Also, abnormal clotting times were found when according to the previous schedule protamine HCl, known to exert multiple effects on the coagulation system, was given frogs (50 mg/100 g). The results show that endotoxin is able to trigger, like in mammals and *Limulus*, the clotting mechanism in frog.