PEPTIDASE ACTIVITY OF AMIDINATED THROMBIN. E. P. Kang. American National Red Cross Blood Research Laboratory, Bethesda, Maryland, U.S.A.

Human thrombin, free of plasminogen and plasmin, was treated with ethyl acetimidate hydrochloride in order to modify the lysyl residues of the protein. By monitoring the enzymic activity in the modification mixture, it was found that the reaction was completed in about one hour and the loss of activity of thrombin was proportional to the amount of modification. After the removal of the excess ethyl acetimidate, approximately 25% of the clotting activity and of the hydrolysis activity for small peptides remained. Amino acid analysis of this modified thrombin indicated about 80% of the lysyl residues had been modified with no apparent change of other amino acid residues. By studying the thrombolytic hydrolysis of Bz-phe-val-arg-pNA, the k_{cat} of the amidinated thrombin was about 8% of the control while the K_M decreased to 0.056 mM from 0.098 mM. The modification of the lysyl residues of thrombin, therefore, has lowered the catalytic efficiency of the enzyme with a rather small change in binding affinity. This suggests that modification of lysyl residues in the neighborhood of the active site hinders the catalytic hydrolysis of the small peptides.

PLATELETS AND MASSIVE TRANSFUSION. J.J. McNamara, H. Leinberger and G. Suehiro, Queen's Medical Center, Honolulu, Hawaii, U.S.A.

Diffuse oozing is a common problem after massive transfusion of stored blood. Coagulation studies usually show depression of all clotting factors, presumably is rarely of sufficient magnitude to cause bleeding. Similarly, platelet counts, which are frequently between 60,000 and 100,000 are still in a range not associated with clinical bleeding. Recent studies have suggested that coagulopathy post transfusion is due primarily to a qualitative defect in platelet function of unknown origin. Five baboons had blood drawn and stored by usual blood banking techniques for 10 days. Platelet rich plasma (PRP) was then prepared and platelets tagged with Cr^{51} . Another five animals had fresh PRP prepared and platelets tagged with Cr^{51} . Survival curves for tagged platelets demonstrated a half life of 13 ± 3 hours for 10-day old platelets and 47 ± 9 hours for fresh platelets. Platelet counts in stored blood averaged 100,000/cmm. Although nearly all 10-day old platelets were cleared by 24 hours, 50% were still present at 13 hours. It is evident then that platelet counts immediately after massive transfusion may be composed in large part of old stored, non-functional platelets.

THE IN VITRO BLEEDING TIME. J. A. Blakely, M. F. X. Glynn and J. Prchal. Dept. of Clinical Hematology, Sunnybrook Hospital, Toronto, Canada.

The observation by Didisheim of platelet-fibrin hemostatic plugs occluding puncture sites in teflon AV shunts prompted an attempt to produce similar plugs *in vitro* using a pump to circulate shed blood. Various anticoagulants, tubing types, flow rates, pressures and methods of producing suitable holes have been investigated. Satisfactory results have been obtained with drilled holes 0.013 - 0.018" diameter in tygon or teflon tubing using heparinized or native, but not EDTA or citrated whole blood. Holes are occluded by platelet fibrin thrombus. Red cells are necessary for plugs to form but are not a component of the plug. Time required for plugs to form correlate with the template bleeding time and are prolonged by thrombocytopenia, aspirin administration, and Von Willebrand's disease. Possible applications are being explored.