P. Friberger, G. Axelsson and K. Korsan-Bengtsen (AB Befors, Peptide Research Laboratory and Department of Internal Medicine II, Sahlgren's Hospital, Göteborg, Sweden): **Determination of Plasminogen by Means of a Chromogenic Peptide Substrate.** (17)

Plasmin splits the chromogenic substrate B2-Phe-Val-Arg-pNA (S-2160, Bofors) at a relatively high rate. Standard plasmin in glycerol obtained from Nat. Inst. for Biol. Stand. and Contr., London, was tested in a system with Tris buffer of varying pH and ionic strength. The pH optimum for the reaction was found to be 7.4. Variations in ionic strength between 0.05–0.1 had insignificant influence but at higher ionic strength there was a slight inhibition. A linear relationship was found between plasmin and ΔOD/min. At optimum pH and a final substrate concentration of 0.2 mM 0.1 CTA unit corresponds to approximately 0.10 nkat. Purified plasminogen (AB Kabi, Stockholm, Sweden) in the concentrations 0.02–0.2 CU/ml was activated optimally with streptokinase (Kabikinase®) in the concentrations 500–2000 IU/ml. Higher concentration gave inhibition. The activity of streptokinase activated plasminogen increased with a decreasing ionic strength. A linear relationship was found between streptokinase activated plasminogen and ΔOD/min. Approximately 3,000 Plong/units per ml of urokinase was needed to obtain the same activation as with optimal streptokinase concentration. The method has been used for determination of plasminogen in plasma. With final dilution of plasma in the range 1/20–1/200 activated by streptokinase (2000 IU/ml) in a system of pH 8.2, I = 0.05, a linear relationship was found between plasma dilution and ΔOD/min. The reproducibility in a series of tests is good (variation coefficient $< 3\%$) and with insignificant interference by inhibitors. The determinations were easily carried out in a simple spectrometer (405 nm) and in an automatic reaction rate analyzer (LKB 8600, 410 nm).

J. C. Giddings, E. Shaw, E. G. D. Tuddenham and A. L. Bloom (University Hospital of Wales, Cardiff. U.K.): **The Synthesis of Factor V in Tissue Culture and Isolated Organ Perfusion.** (18)

Human foetal tissues were maintained in tissue culture and the presence of factor V detected by an indirect immunofluorescence technique. Established monolayer cultures of human foetal liver cells showed positive immunofluorescence when treated with rabbit anti human factor V serum and fluorescein isothiocyanate conjugated with anti rabbit immunoglobulin. Parallel liver cultures containing cycloheximide did not show positive fluorescence when stained by the same technique.

Hepatic factor V synthesis was also investigated by an ex-vivo organ perfusion method using adult rat livers. Factor V synthesis was most clearly demonstrated utilising leucocyte- and platelet-poor perfusate. Factor V synthesis was totally inhibited by cycloheximide and was inhibited by actinomycin D after an initial delay. Factor V synthesis was not inhibited by Warfarin.

The results indicate that factor V is synthesised in the liver by a mechanism similar to that of other plasma proteins and is not dependant on vitamin K for its activity.

C. A. Owen, Jr. and E. J. W. Bowie (Mayo Clinic, Rochester, MN, U.S.A.): **An Artifact in Isolated Organ Perfusion Studies of Coagulation Factors.** (19)

Using isolated rat livers, Mattii et al. (Proc. Soc. Exper. Biol. Med. *116*, 69, 1964) and Olson et al. (J. Clin. Invest *45*, 690, 1966) observed generation of modest amounts of factor V. With rabbit liver-spleen combinations, Dodds and Hoyer (Br. J. Haem. *26*, 497, 1974) found factor VIII emerging. We have perfused rat livers with rat blood cells in bovine albumin-Tyrode's solution. One-stage assays for factors V, VIII, XI and XII indicated release of all 4 clotting activities within the first hour of perfusion, followed by a slow diminution. However, the same occurred if there were no liver in the perfusion system. When the rath erythrocytes were free of platelets ($> 90\%$) and leukocytes ($> 75\%$) before mixing with albumin-Tyrode's, the isolated liver generated factor XII but not factors V, VIII and XI. Production of fibrinogen, prothrombin and factors VII, IX and X was not altered by removing the leukocytes and platelets.