(Dr. Kirby, Temple University provided the purified factor VIII's and Prof. Perkins, Liverpool University provided ALAA; supported by N. I. H. HL 14217.)

J. Stibbe and E. Kirby (Spec. Center for Thrombosis Research and Dept. of Biochemistry, Temple Univ., 3400 N. Broad St., Philadelphia, Pa. 19140 and Erasmus Univ., Rotterdam): **Inhibition of Ristocetin-Induced Platelet Aggregation (RIPA) by Haemaccel® and Fibrinogen.** **(257)**

RIPA was investigated using purified human factor VIII (F VIII) and formalin-treated washed platelets suspended in tris buffered saline. At constant (F VIII), aggregation was very sensitive to the conc. of Ristocetin (R) in the range 0.2–0.5 mg/ml, but higher (R) did not further increase aggregation. At constant (R), increasing (F VIII) caused increased aggregation for any (F VIII) used (0.3–3.0 μg protein/ml, 0.015–0.15 U F VIII-related antigen/ml).

RIPA was inhibited by low conc. (0.15 mg/ml) of the gelatin plasma expander Haemaccel (H) (Behringwerke). Addition of H after aggregation was complete caused reversal of the aggregation. The inhibition could be overcome by increasing (R), but not by increasing (F VIII) in the conc. ranges used. The conc. of R which overcame H inhibition also caused precipitation of H. This precipitation could be reversed by increasing (H). Our evidence suggests that H inhibits RIPA by binding R, thus inhibiting the interaction of R and F VIII on the platelet surface which is responsible for aggregation.

Human Fibrinogen (Fb) (Kabi, 99% pure, 0.5 U F VIII-related antigen/mg) caused a similar inhibition of RIPA in conc. as low as 0.2 mg/ml. Higher conc caused greater inhibition, which could be overcome by increasing (R). The Fb (2.5 mg/ml) itself did not induce aggregation in the presence of R. R may also bind to other plasma proteins as well since it can form a precipitate with serum.

These findings are of considerable importance for the quantitative measurement of the Ristocetin co-factor. RIPA is very sensitive to a small range of (R) and the availability of R in the test system can be greatly affected by the presence of Fb and other plasma proteins.

M. Blombäck and N. Egberg (Karolinska sjukhuset, S-104 01 Stockholm, Sweden): **Two Cases of von Willebrand's Disease with Specific Inhibitors of Ristocetin Induced Aggregation of Normal Platelets.** **(258)**

Ten patients (8 females and 2 males) with severe form of von Willebrand's disease were investigated for the occurrence of inhibitors blocking Ristocetin induced aggregation of normal platelets. All these patients had been multiply transfused and had the classical signs of von Willebrand's disease, prolonged bleeding time, less than 10% of normal factor VIII activity and correspondingly low factor VIII antigen levels, normal ADP induced platelet aggregation but no Ristocetin induced aggregation. In none of these patients circulating anticoagulants neutralizing factor VIII activity had been demonstrated. Plasma samples from 2 of these patients were found to block Ristocetin induced platelet aggregation of normal plasma. In one of these patients the inhibitor was found to be of IgG type. This patient otherwise had a normal serum electrophoretic pattern. The inhibitor of the other patient is being investigated.

F. R. Matthias, R. Reinicke, D. L. Heene and H. G. Lasch (Dept. of Int. Medicine, Justus Liebig-University, Giessen, Germany): **Determination of Fibrinmonomer (FM) from Plasma by Affinity Chromatography on Insolubilized Fibrinogen (FG-ag).** **(259)**

Quantitative estimation of soluble fibrinmonomer in plasma may be achieved by affinity chromatography on insolubilized fibrinogen (FG-ag), as described by us (Heene, Matthias: Thrombosis Res. *2*, 137, 1973). The procedure is based on the reversible adsorption of fibrinmonomer to FG-ag. Column: 0.9×28 cm FG-ag; sample volume: 2 ml plasma; adsorption buffer A: 0.05 M tris-H_3PO_4, 0.1 M NaCl, 0.001 M TAME, 0.005 M