Citrated plasma (10 µl) was diluted in 600 µl tris buffer pH = 8.2 µ 0.15 and activated with 250 µl of a commercial rabbit brain-lung thromboplastin (Simplastin, General Diagnostics Division, Warner-Lambert, Morris Plain, U.S.A.) After 5 minutes incubation at 37° C 200 µl of a 1 mM solution of the chromogenic substrate was added and the increase in optical density was recorded in a LKB-Beckman 8600 enzyme analyzer. A reading time of 1 minute was used which permitted 60 analyses per hour to be carried out. A linear relationship was found between ΔOD and dilutions of normal plasma in prothrombin deficient plasma or adsorbed plasma. The methcd is insensitive to variations in factor V, VII and X. Less than 5% of normal plasma was needed to "normalize" plasmas deficient in factor V or VII or X. The method can be used to control dicumarol treatment. A group if dicumarol treated patients has been investigated with the present method and with Quick's prothrombin time and with a P & P-method using Simplastin A (General Diagnostics Division, Warner-Lambert, Morris Plains, U.S.A.) as a reagent. The results of this comparison is presented.

H. Hassouna, J. Reuterby, D. Walz, D. Hewett-Emmett and W. H. Seegers (Wayne State University, School of Medicine, 540 E. Canfield, Detroit, Michigan, 48201, U.S.A.): **Quantitative Determination of Prothrombin and its Fragments in Plasma and Serum.** **(507)**

A comparison of immunochemical and biolcgical methods for quantitative determination of human and bovine prothrombin and its activation fragments in fresh and stored human and bovine plasma and serum is presented. High titered monovalent antisera to prothrombin and specific antisera to PR and 0 fragments produced in rabbits were characterized by radio immunoelectrophoresis and the Feinberg technique using I^{125} labelled antigens. Prothrombin and PR antisera neutralized prothrombin activity of plasma when tested by the prothrombin clotting assays, while bovine 0 fragment was found bound to Ac-globulin in plasma adsorbed with barium carbonate. Antibodies insolubilized by coupling to Bio Gel A-15^{M} were used to deplete fresh plasma of corresponding protein. Depleted plasma was reconstituted by adding varying dilutions of fresh plasma or I^{125} labelled antigens, and amounts of antigen needed to restore its property to form a standard clot was estimated by the Laurell "rocket" technique, and by hemagglutination inhibition. The purpose of the study is to select a suitable quantitative immunoassay to measure antigenically active prothrombin and its fragments in plasma and serum of normal men, women and children grouped according to estimated blood pressure, age and weight.

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R. Gonggrijp and H. C. Hemker (Department of Biochemistry, Medical Faculty Maastricht, The Netherlands): **The Activation of Factor VII.** **(508)**

Native factor VII (F. VII_n) can be activated by thromboplastin or by the kallikrein-system. This gives rise to two different forms of activated factor VII. We thus discern Factor VII_n, cold activated factor VII (C.P.A.-F. VII_a) and thromboplastin activated factor VII (T-F. VII_a).

We developed one- and two-stage assays that distinguish between these forms and we tried to establish their interrelationship.

T-F. VII_a could be obtained in a form devoid of lipid components and still clearly distinguishable both from factor VII_n and from C.P.A.-F. VII_a. It activates factor X in a thromboplastin-independent process, whereas C.P.A.-F. VII_a is unable to do so.

The difference between F. VII_n and C.P.A.-F. VII_a is that the latter is converted into T-F. VII_a much more quickly in a process that shows less species specificity with regard to the type of thromboplastin used.

No differences could be seen between T-F. VII_a obtained from V. VII_n directly or via C.P.A.-F. VII_a.