DETECTION OF HEMOPHILIA A CARRIERS BY REPEATED FACTOR VIII ACTIVITY/ANTIGEN DETERMINATIONS. U. Seligsohn, A. Zivelin, H. Peretz and M. Modan. Sheba Medical Center, Tel-Hashomer, Israel.

Detection of hemophilia A carriers (C), using factor VIII (fVIII) activity (Ac) and antigenicity (Ag) measurements, has been hampered by an overlap of results obtained in normal females (NF) and obligatory carriers (OC). In an attempt to improve the specificity and sensitivity of [illegible] and 33 NF were examined 3 times. FVIII Ac and fVIII Ag were determined in fresh plasma samples, employing a one stage method and the Laurell technic with rabbit anti fVIII antiserum, respectively. Using the mean of the 3 pairs of measurements, a discriminant function, based on Bayes' theorem, was obtained for calculating the probability that a woman is a C. Three ranges of measurements were defined: Definite C, possible C and definite NF. The results were as follows:

Range	OC	NF
Definite NF	3.4%	87.9%
Possible C	20.7%	12.1%
Definite C	75.9%	0

If only the first pair of measurements was used, the results were much less satisfactory:

Range	OC	NF
Definite NF	6.9%	81.8%
Possible C	34.5%	18.2%
Definite C	58.6%	0

Thus, for each suspected C, the relative odds of being a C can be assigned by using this function, but the results should be based on the mean of 3 measurements.

THE ISOLATION OF A POTENT PLASMA KALLIKREIN WITH WEAK PLASMINOGEN ACTIVATOR ACTIVITY. M.J. Gallimore, E. Fareid and H. Stormorken. Institute for Surgical Research , Institute for Thrombosis Research, Rikshospitalet, Oslo, Norway.

Kallikrein was isolated from human plasma by the following procedures: removal of euglobulins at pH 5.3; QAE Sephadex chromatography; gel filtration on Sephadex G-150 and G-100. The partially purified preparation was then freed of contaminants by running it down a column of Sepharose-4B to which had been linked antibodies to IgG and pre-PTA. Pre-kallikrein and kallikrein activities were monitored during the fractionation procedures using a new synthetic chromogenic substrate for plasma kallikrein (Chromozyme-PK, Pentapharm, Basle, Switzerland). 5 mg of enzyme was obtained with a specific activity of 3.75 Chromozyme PK (CPK) units/mg at 22° (yield = 9.5%; purification factor = 6250) and the yield of kinin from heated plasma was 1.79 µg/CPK unit/min. The kallikrein exhibited very weak plasminogen activator activity when tested on fibrin plates and in fibrin clot lysis assays (1 CPK unit = 0.133 CTA units urokinase). Some other properties of the enzyme are discussed.

ARTIFICIAL SUBSTRATE PLASMA FOR THE SPECIFIC ONE-STAGE FACTOR X ASSAY. H. Beeser and K. Kulzer. Institute for Experimental Hematology, University of Bonn, Fed. Rep. Germany.

As patients who are congenitally deficient in factor X are extremely rare natural substrate plasma for the specific one-stage factor X assay is only poorly available. Asbestos-filtered or charcoal-filtered ox plasma deficient both in factor X and VII is instead widely used as artificial factor X substrate plasma with Russel's viper venom (RVV) and cephalin or lecithin as the thromboplastic agent.

We studied the practicability of removing separately factor X from the plasma of different species (man, horse, ox, sheep, swine) by several chromatographic procedures. Chromatography of swine plasma on DEAE-Sephadex A 25 yielded a substrate virtually free of factor X while containing adequate concentrations of fibrinogen and factor II, V and VII. Using different brands of commercial tissue thromboplastins the so prepared swine plasma has proved to be a specific artificial substrate plasma for the one-stage factor X assay. Calibration curves obtained by plotting the clotting times of various dilutions of pooled normal human plasma determined with this factor X assay system against the percentages showed a linear relationship from 100 % to about 2 % on log/log paper. Factor X determinations with our method on various patients' plasmas (i.e. coumarin treatment, congenital factor X deficiency, liver disease) performed in parallel to the RVV-cephalin and the congenital factor X deficient plasma methods gave comparable results.

As our specific artificial factor X deficient swine plasma is fairly simple prepared with a good yield, it has to be considered as an appropriate reagent to overcome the shortness of the rare congenital factor X deficient substrate plasma.