Time

09.15 **0866** DEMONSTRATION OF COMPLEXES BETWEEN FACTOR IX AND IGG ANTIBODIES FROM HEMOPHILIA B PATIENTS

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Plasma from three patients with hemophilia B- and an acquired inhibitor to factor IX (titres: 5 U/ml, 0.73 U/ml and 0.32 U/ml) were incubated with purified factor IX and submitted to crossed immunoelectrophoresis against a rabbit antiserum to factor IX. A heterogenous precipitin arc was seen instead of the homogenous precipitin arc seen when a control plasma from a patient with hemophilia B- and no acquired inhibitor was used for the incubation. Human IgG antibodies were demonstrated in the cathodal part of the precipitin arcs. This was achieved by incubation of the agarose gels, after the electrophoresis, with a peroxidase conjugated rabbit antiserum to human IgG. The IgG antibodies from the two patients with the highest inhibitor titre contained both kappa and lambda light chains, and were thus of a polyclonal nature. It is concluded that IgG antibodies from low-titred as well as high-titred inhibitor plasmas form complexes with factor IX. The detection of IgG in complex with factor IX may be a sensitive technique for the detection of low-titred inhibitors to factor IX.

09.30 **0867** BINDING OF ALLO- AND HETEROANTIBODIES TO HUMAN FACTOR IX (F.IX)

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Study of the binding properties of human alloantisera to F.IX is made difficult by the nonprecipitating nature of the antibodies. Most studies have relied on F.IX coagulation neutralization to characterize such immune inhibitors. We have directly measured the binding of antibodies to highly purified radiolabelled F.IX (^{125}I-F.IX) by using either differential precipitation [employing ammonium sulfate (AS) or polyethylene glycol (PEG)] or column chromatography to separate antigen-antibody complexes from free ^{125}I-F.IX. The two heteroantisera and four immune alloantisera studied all form stabile immune complexes with ^{125}I-F.IX in vitro. The various antibodies require different levels of AS or PEG for optimal precipitation of complexes. Precipitability is not related to antibody titer as measured by radioimmunoassay or coagulation inhibition but may be related to the size of the immune complex. Using column chromatography on agarose A-5m, two alloantisera studied form complexes with molecular weights of less than 5×10^5 daltons. The low molecular weight of such complexes suggests that a limited number of antigenic sites on the F.IX molecule are recognized by alloantisera.

09.45 **0868** ACTIVATION OF NORMAL AND ABNORMAL (GENETIC VARIANT) HUMAN FACTOR IX

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Isolated human factor IX (single chain; MW_{HC} 63,000) has been activated in three ways:
a) in the presence of factor XIa and Ca^{2+} active 2-chain factor IXa (MW 47,000; MW_{HC} 27,500) is formed via an inactive 2-chain intermediate (MW 63,000; MW_{HC} 44,500);
b) in the presence of thromboplastin, factor VII and Ca^{2+} essentially the same sequence of reactions takes place as sub a;
c) in the presence of RVV-X active 2-chain factor IXa (MW 63,000; MW_{HC} 30,000) is formed.
The dependance of these three reactions on the Ca^{2+} concentration has been studied.

A genetic variant of factor IX was found that like PIVKA IX can be separated from normal factor IX by electropheresis in the presence of Ca^{2+}. Again like PIVKA IX this variant shows a strongly reduced affinity for binding to $Al(OH)_3$. These findings suggested an abnormality in the Ca^{2+} binding properties of this variant.

The activation of the isolated variant factor IX by the forementioned activators will be compared with that of normal factor IX.

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