0559
08:15 h

THE STRUCTURAL ERROR AND ITS RELATION TO THE MALFUNCTION IN SOME ABNORMAL FIBRINOGENS. A. Henschen, C. Southan, M. Kehl and F. Lottspeich. Max-Planck-Institut für Biochemie, Martinsried/München, GFR.

The primary structure elucidation of normal human fibrinogen has recently been completed. Thus, it is now possible to analyse the structure-function relationship in detail. In abnormal fibrinogens the function is altered. It is obvious that information about the corresponding structural errors would be of great importance. Functional abnormality of fibrinogen has been described for more than 70 cases. However, only in a single case, Fibrinogen Detroit, the structural error was identified by Blombäck's group. Recently, our group has analysed several abnormal fibrinogen by separating the three peptide chains and performing direct sequence analysis of the chains. The first fibrinogen (obtained from R. Marx and W. Schramm) had an Arg→Asn substitution in position 19 of the Aα-chain, i.e. in the same position as in Fibrinogen Detroit, and was characterised by delayed A-peptide release and monomer polymerisation. The second fibrinogen (obtained from C. and J. Soria) had an Arg→Cys exchange in position 16 of the Aα-chain, and was characterised by absence of releasable A-peptide. The third fibrinogen (obtained from V. Hofmann) had the same substitution as the previous, but in the heterozygous state, and was characterised by delayed A-peptide release. It is obvious that when the Arg residue in position 16, i.e. at the thrombin cleavage site, is replaced by a neutral residue thrombin will not cleave. When the Arg residue in position 19 is exchanged the cleavage is slowed down. Several other fibrinogens with delayed A-peptide release showed, however, no primary structure error in this section of the molecule.

0560
08:30 h

CARBOHYDRATE COMPOSITION AND CATABOLISM OF FIVE ABNORMAL FIBRINOGENS. D.A. Lane, A.K. Allen, J. Markwick, I. Mackie, E. Thompson and J. Owen. Departments of Haematology and Biochemistry, Charing Cross Hospital Medical School and Department of Haematology, Central Middlesex Hospital, London, U.K.

An investigation has been made of the carbohydrate composition and catabolism of five abnormal fibrinogens. These were fibrinogens, London, Manchester, Oslo II, a fetal fibrinogen isolated from pooled cord blood and a newly discovered case of hypodysfibrinogenemia which has been tentatively designated fibrinogen London II. The carbohydrate composition of fibrinogen (mol sugar/mol of fibrinogen) was studied by amino acid analysis techniques for amino sugars and methanolysis followed by GLC for neutral sugars and sialic acid. The adult variants did not differ significantly from the normal adult fibrinogen which typically contained 22 mannose, 18 galactose, 20 N-acetylglucosamine, 4 N-acetylgalactosamine and 12 sialic acid residues. However, fetal fibrinogen was markedly different, containing 15 mannose, 21 galactose, 14 N-acetylglucosamine, 20 N-acetylgalactosamine and 20 sialic acid residues. The catabolism of the I^{125}-labelled fibrinogens was studied in New Zealand White rabbits. Normal fibrinogen was eliminated with a mean $t\frac{1}{2}$ of 50.2 h (n = 5, range 42-60). The mean $t\frac{1}{2}$ of fetal fibrinogen was similar 47.7 h (n = 5, range 41-70) and fibrinogens London, London II, Manchester and Oslo II had $t\frac{1}{2}$'s of 39, 42, 38 and 44 h respectively. It is concluded that (a) the increased sialic acid on fetal fibrinogen is probably due to there being more O-glycosylated serine or threonine residues and this does not alter catabolism, (b) the reduced plasma fbgen of London II is caused by impaired synthesis rather than hypercatabolism, and (c) the catabolism of fibrinogen is only minimally dependent upon the full integrity of the regions of the molecules involved in polymerisation and fibrinopeptide releasing functions.

0561
08:45 h

ISOLATION AND CHARACTERIZATION OF FRAGMENTS OF HUMAN FIBRINOGEN GENERATED BY CHEMICAL CLEAVAGE AT CYSTEINE RESIDUES. Y. Benabid and R. F. Doolittle, Department of Chemistry University of California, San Diego, La Jolla, CA 92093 USA

Human fibrinogen and its constituent S-sulfo chains were treated with 2-nitro-5-thiocyanobenzoic acid (NTCB) under conditions appropriate for cleaving on the amino-terminal sides of all cysteine residues. The fibrinogen or chains were first reduced with dithiothreitol (DTT) and the excess reducing agent removed by dialysis under nitrogen. The course of fragmentation was followed by SDS polyacrylamide gel electrophoresis and the purification of major fragments accomplished by gel filtration under a variety of solvent conditions. Our primary aim was to isolate and study the 112/113-residue polypeptides that exist between disulfide rings in the native molecule and that are thought to comprise the "coiled-coils" that connect the central and terminal domains. We also attempted to purify the other major fragments produced by NTCB-treatment, however, including 277-residue and 139-residue fragments from the α-chain, and a 108-residue β-chain polypeptide and a 144-residue fragment from the γ-chain. Of all of these, the 277-residue peptide from the α-chain is the easiest to isolate and was readily obtained in pure form. The 112/113-residue interdomainal connectors presented a number of technical problems with regard to their isolation, including a decided propensity to self-associate. Accordingly, we resorted to circular dichroism as a tool for following the residual α-helix that is an index of the presence of these segments, a technique we had planned to use to study the re-association of the separate segments. Preliminary evidence suggests that we were able to reform α-helical structures, presumably coiled-coils, to various degrees with different combinations of segments from the different chains.

0562
09:00 h

SEQUENTIAL FORMATION OF DES-A FIBRIN AND DES-AB FIBRIN AND BINDING OF FIBRINOGEN TO DES-AB FIBRIN. G. Müller-Berghaus, J.-C. Bernhard, H. Hofmann, I. Mahn, F.R. Ostheimer, E. Selmayr and W. Thiel. Department of Medicine, Justus-Liebig-Universität, Giessen, W. Germany.

Thrombin cleaves the fibrinopeptides A and B from fibrinogen in a consecutive manner, producing first des-A fibrin and consequently, des-AB fibrin. Physico-chemical and in-vivo properties of both types of fibrin were comparatively studied by gel filtration, affinity chromatography and in-vivo behavior. At 37°C and in the presence of fibrinogen and Ca^{++}, des-A fibrin formed soluble fibrin-fibrin dimers. Des-AB fibrin, however, formed oligomers at 37°C. Fibrin-fibrinogen interactions were studied by affinity chromatography. At 37°C, des-A fibrin did not interact with immobilized fibrinogen, whereas des-AB fibrin stuck to it. Small amounts of des-A fibrin iv injected were cleared from the circulation much faster than des-AB fibrin. Large amounts of des-A fibrin injected into animals aggregated to microclots. These results suggest that des-A fibrin in a low concentration (0.01 mg/ml) does not form circulating oligomers and can very fast be cleared from the circulation possibly because of its weak interaction with fibrinogen. Aggregated des-A fibrin, however, is cleaved to form des-AB fibrin. Des-AB fibrin binds fibrinogen making it accessible to further thrombin cleavage. Thus, the threshold of clot formation would be the concentration of des-A fibrin in plasma. The preferential sequence of reactions is: fibrinogen → des-A fibrin → des-A fibrin aggregates → des-AB fibrin aggregates. Des-AB fibrin + fibrinogen → des-AB fibrin-fibrinogen → des-AB fibrin-des-A fibrin etc.