PROBLEMS OF QUANTITATION OF F VIII:C ACTIVITY. I. M. Nilsson. T. Barrowcliffe. T. Kirkwood.
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The establishment in 1970 of the first international standard for F VIII by the expert committee on biological standardization of the WHO achieved a major step towards uniform measurement of F VIII:C activity. This has allowed different laboratories to make detailed comparisons of their assay systems. These studies, which include the recently completed international collaborative study of the proposed 2nd I S have repeatedly shown that systematic differences between laboratories still remain. In particular, two-stage assays are detecting relatively more activity, on average, in the more purified preparations than one-stage assays. The discrepancy between methods may be as high as 20-25 %. Also, even with the same methods, different sets of reagents are giving substantially different results. The fundamental characterization required of a F VIII preparation is a measure of its in vivo effect. Present understanding of F VIII biochemistry suggests that this effect may be the product of complex interactions between several different activities. Nevertheless the world price of F VIII preparations in determined largely in terms of units of clotting activity. The discrepancies between assay systems thus have significant financial as well as clinical and scientific implications. This suggests that international agreement should be sought as to which assay systems may be relied upon to give realistic measures of potential in vivo effect.

# POSTER SYMPOSIUM IV

## Coagulation: Primary Structure of Fibrinogen.

ON THE PRIMARY STRUCTURE OF HUMAN FIBRINOGEN. 
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The aim of the present investigation is to elucidate the primary structure of human fibrinogen. Through the work of several laboratories including our own large parts of the structure are now known. Here will be reported the complete amino acid sequence of the γ-chain (409 residues). Furthermore, the carbohydrate linkage site in the β-chain and plasmin cleavage sites in the β- and γ-chains have been identified.

The peptide chains were isolated by CM-cellulose chromatography following mercaptolysis and alkylation. The γ-chain was cleaved in a way to produce large fragments suitable for automatic sequencing, e.g. with cyanogen bromide or trypsin after citraconylation. The sequences of the isolated fragments allowed reconstruction of the complete sequence of the γ-chain.

The carbohydrate linkage site in the β-chain could be isolated by affinity chromatography on concanavalin A-agarose following cleavage of the chain by trypsin or cyanogen bromide. The sequence of 21 amino acid residues around the carbohydrate attachment site was determined.

The plasmin cleavage site giving rise to N-terminal glycine in the γ-chain D-fragment was identified. The amino acid sequence linking plasmic fragments E and D in the β-chain was determined.