PURIFICATION AND PARTIAL CHARACTERIZATION OF RAT FIBRINOGEN (rat-Fbg). W. Nieuwenhuizen and Irina A.M. van Ruijven-Vermeer. Gaubius Institute, Health Research Organization TNO, Herenstraat 5d, Leiden, the Netherlands.

Rat-Fbg was purified from rat plasma using Sepharose-lysine chromatography, repeated ammonium sulphate precipitation (35% saturation) and gel chromatography on Sepharose 6B.

In order to minimize proteolytic activity, rats were injected intravenously with Trasylol before bleeding, and the collected blood was treated with Trasylol and DFP.

A preparation was obtained, which was 95% clottable and showed a single band on SDS-polyacrylamide gel electrophoresis. Alanine was the only detectable amino-terminal amino acid.

After reduction and modification of the SH groups the material could be separated into three distinct chains (Aα, Bβ and γ) by pore-limit polyacrylamide slab-gel electrophoresis in sodium dodecyl sulphate. The amino acid composition of the whole Fbg and of the separated modified chains were determined. The molecular weights were 61,000, 58,000 and 51,000 for Aα, Bβ and γ-chains, respectively.

In as far as the chains are concerned, our results are in contrast with the findings of Bouma et al. (J.Biol.Chem. 250(1975) 4678), who could not discriminate between Aα- and Bβ-chains in SDS-polyacrylamide gel electrophoresis. Evidence will be presented that this can be due to Aα-chain degradation caused by incomplete inhibition of proteolytic enzymes during the purification.

It is concluded, that complete inhibition of proteolytic activities in all purification steps is essential to obtain native fibrinogen. Moreover, in contrast to the conclusions of Bouma rat-Fbg does not differ essentially from Fbg from other mammalian species.

NON-SPECIFIC BINDING OF THIOCHOLINE ESTER OF CINNAMIC ACID TO FIBRIN. T. Seelich, B.A. Perret, M. Furlan and E.A. Beck. Central Hematology Laboratory, Inselspital, Berne, Switzerland.

Thiocholine esters inhibit the enzymatic crosslinking of fibrin (1). Thiocholine ester of ^{14}C-labelled cinnamic acid (2-diethylbenzylaminoethyl thioltranscinnamate bromide) was prepared and incubated with human fibrinogen in the presence of factor XIII and thrombin. Polyacrylamide gel electrophoresis with sodium dodecyl sulphate, followed by liquid scintillation counting of the separated protein bands, showed that all three chains of fibrinogen had bound the crosslinking inhibitor (2 moles/mol α chain, 1.5 moles/mol β chain, 2.2 moles/mol γ chain). Cyanogen bromide cleavage of the isolated α chain resulted in 6 major fragments all of which were radioactive. These results indicate that the binding of the thiocholine ester of cinnamic acid was not restricted to the specific donor crosslinking sites of fibrin(ogen). This thiocholine ester differs in this respect from dansylcadaverine which binds exclusively to the acceptor sites involved in fibrin(ogen) crosslinking.

(1) Lorand et al., Proc. Nat. Acad. Sci. 69, 2645, 1972.

FIBRINOGENS OF COMMON LABORATORY MAMMALS - A COMPARATIVE STUDY. R.J.Hawker and Linda M. Hawker. Department of Surgery, University of Birmingham, Birmingham, U.K.

Many laboratory mammals are used as in vivo or in vitro models of haemostatic mechanisms and require readily available purified fibrinogen suitable for trace labelling. A comparative study was made on the fibrinogens extracted by a standard 45 minute procedure from man and the following common laboratory mammals: baboon, rhesus monkey, dog, cat, rabbit, guinea pig, rat and the mini-pig. The method* utilising 5.5 mls of plasma is applicable for autologous preparations and gives 60-70% yields of pure fibrinogen from all species being dependent upon the fibrinogen concentration of the starting plasma. Clottability was consistently in excess of 90% and the final solution was suitable for direct labelling with 125-Iodine using Chloramine T.

The fibrinogens were examined for purity on sodium dodecyl sulphate polyacrylamide gels from more than 60 different individuals, using reduction with mercapto-ethanol to compare the subunit molecular sizes (Aα Bβ γ). No other proteins were detectable nor were enzymatically derived modifications. We demonstrate molecular size differences as high as 9,000 daltons between the same designated subunit within a phylogenetic order, for example, the Aα subunit of the non-human primates compared with that of man.

* Hawker, R.J. and Hawker, Linda M. 1976. J. Clin. Path. 29, 495-501.