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0472 REMOVAL OF POTENTIALLY THROMBOGENIC MATERIAL FROM A CONCENTRATE OF FACTORS II, IX & X BY POLYETHYLENE GLYCOL PRECIPITATION

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Polyethylene glycol (PEG) precipitation has been used for the removal of hepatitis B surface antigen (HBsAg) from a concentrate of factors II, IX & X of intermediate purity. HBsAg is precipitated into a fraction P1 at 20% (w/v) PEG and pH 6.5 while factors II, IX & X remaining in the supernatant are subsequently precipitated at 30% (w/v) PEG and pH 5.2.

These various fractions have been assessed for the presence of potentially thrombogenic materials by *in vitro* tests. In comparison with the starting material the precipitate rich in factors II, IX & X has a significantly increased non-activated partial thromboplastin time (NAPTT) and a decreased rate of factor Xa generation. By contrast the waste fraction (P1) exhibits a significantly decreased NAPTT and an increased rate of factor Xa generation suggesting that potentially thrombogenic material has been selectively precipitated. This procedure has been used to prepare a factor II, IX & X concentrate containing 100 U FIX/ml with reduced contamination by both HBsAg and thrombogenic materials.

## Platelet Proteins

Level 6 - Red Side

Discussion Group 12.00 - 12.45

P6-080 0473 CHARACTERIZATION OF PIG PLATELET GRANULE PROTEINS

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Platelet granules contain glycoproteins similar to those found in platelet membranes (Hagen et al, BBA, 445, 214, 1976). Pig platelet granule fractions enriched in mitochondria,  $\alpha$ -granules or dense granules were analyzed by SDS polyacrylamide gel electrophoresis to determine if there are differences among the organelles. In a reduced system (5% DTT) the proteins of the  $\alpha$ -granules and dense granules showed staining patterns with Coomassie blue that were distinctly different from whole platelets, isolated membranes or mitochondria. In the granules about 10 to 12 bands with less mobility than actin were visualized. Staining with PAS was obtained in bands with apparent molecular weights of 250, 225, 185, 170, 150, 120, 55, 48 and 40 K. The 185 K band appeared to be the same as "thrombin sensitive protein". The mobility of the 55 and 48 K bands were identical with the B ( $\beta$ ) and  $\gamma$ -bands of bovine fibrinogen. The PAS staining of the granule components was more intense than that of whole platelets for the same amount of protein, indicating that granule membranes may be as rich in glycoproteins as external plasma membranes. With both PAS and Coomassie blue, the  $\alpha$ -granule and dense granule staining patterns were almost identical. This observation may be relevant to recent studies which showed that both granule types exhibited similar release characteristics, suggesting that they share a common release mechanism. NIH-USPHS Grant No. 14217