- A high freezing and thawing rate of the plasma results in a high recovery of factor VIII and less contaminating proteins and lipids in the cryoprecipitate.
- After centrifugation and dissolving of the precipitate, the preparation may be lyophilized. An appreciable loss of activity is found when the temperature during this process exceeds 20° C.
- From the cryoprecipitate factor VIII can be further purified by precipitation with PEG 4000. The fraction between $2^1/_2$% at 20° C and 6% at 0° C was used. Within certain limits this precipitation is independent on the protein concentration, pH, time, and ionic strength. Mandelate (0.3% w/v) prevents irreversible precipitation of fibrinogen during all operations.
- Sterilization of the dissolved precipitate was achieved by filtration through membrane filters (0.3 μ) on which the filter aids Celite 535 and Hyflo supercel were layered.
- PEG precipitation and sterilization induce a 20% loss.
- The clinical use of the product was assayed in series of infusions in hemophiliacs.

J. F. Davidson, J. H. McAdam, M. J. Mackenzie, M. L. Kavanagh (Department of Haematology, Glasgow Royal Infirmary, Glasgow, U.K.): **Improved Factor VIII Yield in Cryoprecipitate Using a Quick Thaw Technique.** **(276)**

Standard Cryoprecipitate was prepared from fresh citrate phosphatedextrose plasma by snap freezing at —70° C and then thawing at +4° C in air for 18 hours. In 143 experiments the yield of Factor VIII from the starting plasma was 42%.

In 64 paired experiments the Factor VIII yield in Cryoprecipitate from fresh plasma was increased, from 43% in the standard method to 56% when a quick thaw of 50 minutes at +4° C in a liquid bath was introduced. In 10 other paired experiments the yield in the standard method was raised from 51% to 61% when 90 minutes of super-cooling at —6° C in a liquid bath was introduced prior to snap freezing. When, however, the quick thaw and super-cooling modifications were combined in 42 paired experiments, the yield was only 49% compared with 42% by the standard method.

It is concluded that this simple quick thaw modification will produce a greater yield of Factor VIII in Cryoprecipitate and that the addition of the technically more demanding super-cooling modification does not give a significantly greater yield.

It seems likely that the longer period at +4° C in the standard method leads to denaturation of a proportion of the Factor VIII and loss of activity. Factor VIII antigen, however, was not lost. In a smaller number of experiments approximately all the Factor VIII was recovered in the Cryoprecipitate and its supernatant. Furthermore, the relative proportions of Factor VIII antigen and procoagulant in the Cryoprecipitate were found to vary in concert suggesting that the Factor VIII molecule is not dissociated in the process of cryoprecipitation.

Michael R. Downing, Ralph J. Butkowski and K. G. Mann (Hematology Research, Mayo Clinic, Rochester, MN. 55901 U.S.A.): **Human Prothrombin Activation Products.** **(277)**

SDS gel electrophoretic analysis of the products of human prothrombin (II_H) activation reveal a similar product distribution to that observed for bovine prothrombin (II_B). However, some subtle differences are noted when the amino-terminal sequences of the activation intermediates are determined. The amino-terminal sequences of II_H and II_H-intermediate 3 are identical (ALA, ASN, PRO, PHE, LEU, GLU, GLU, VAL, ARG, LYS) and homologous to the sequence for II_B. Similarly the amino-terminal sequences of II_H-intermediate 1 and II_H-intermediate 4 (SER, GLU, GLY, SER, SER, VAL, ASN, LEU, SER, PRO) are entirely homologous with their bovine counterparts. On the other hand II_H-intermediate 2 and α-thrombin A chain have the sequence THR, PHE, GLY, SER, GLY, GLU, ALA, ASN and this sequence is homologous to the II_B intermediate 2, α II_a A chain beginning with residue 14. Studies of the cleavage of II_H-intermediate 1 with factor X_a in the presence of DFP and Hirrudin and with thrombin in the presence of soybean trypsin inhibitor indicate that the II_H intermediate 2 originally produced by