FROM DONOR TO FRACTIONATOR:

HOW IS FACTOR VIII LOST?

A Literature Survey

A short review of the literature is given under the following headings:

1. Donation Time and Mixing
2. Anticoagulant
3. Centrifugation and Cellular Contamination
4. Temperature and Time from Donation to Freezing
5. Freezing Rate
6. Frozen Temperature and Storage Time
7. Factor VIII in Feedstock
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1. DONATION TIME AND MIXING

Basic experiments show blood collected in plastic equipment does not clot for 10 to 15 minutes and that incipient clotting as detected by fibrinopeptide A (FpA) generation does not occur for 5 minutes (Prowse 1980). Early work suggests somewhat longer donation times (10 to 15 minutes; Vermeer 1976b, Preston 1967) for plastic packs than glass bottles (4 to 5 minutes, Goldstein 1964) but more recent data shows an 8 minute limit is now more usual (Newman 1971) and an average of 4 to 5 minutes was found in a local study with only 10 per cent of donations exceeding 8 minutes (SBTS 1983). Attempts to show differences in factor VIII between fast and slow donations have failed (Preston 1967, EBTS 1974) except where minor or overt clotting occurs (Hondow 1982b) but in these studies most donations were collected in under 10 min. Preston (1967) even found that delaying addition of anticoagulant until 9 minutes had no effect. Exposure to low pH (< 5.8) during the initial exposure of blood to ACD anticoagulant may result in a loss of about 10% of factor VIII (Preston 1967, Vermeer 1976b), although Goldstein (1964) failed to observe this, an effect which may be reduced by faster donation, increasing anticoagulant pH (CPD) or lowering temperature.

Although Perkins (1962) showed a 15% factor VIII difference between factor VIII in ACD blood collected with and without mixing and Penick (1956) suggested a greater lability of factor VIII in unmixed blood, subsequent experiments have failed to show any such difference (Preston 1967, Slichter 1976, EBTS 1974). Kasper (1975) found fresh plasma obtained from donations obtained on the Hemolater, mixed 40 or once per minute or unmixed all contained about 0.9u VIII/ml and only found a 20% loss in blood which was collected without mixing and then left layered on the anticoagulant for 4 hours prior to centrifugation.

Using FpA rather than VIII assay Pflugshaupt (1981) has shown mixing improves plasma quality in terms of processability, but not factor VIII levels. A local study (SBTS 1983) failed to confirm this effect and Myllyla (1983) states that very bad handling is required to obtain the levels given by Pflugshaupt. The local study did confirm donation time in excess of 10 min or delayed stripping of donation lines did result in raised fibrinopeptide levels (SBTS 1983). More recently Pflugshaupt (1981) has used a pump allowing metered addition of anticoagulant during donation and claims this improves both FpA and factor VIII levels. This is a prototype pump only.

RECOMMENDATION

1. An 8 to 10 minute limit for blood donation is established and acceptable.

2. Current Scottish standards for mixing during donation appear to be adequate.

3. Purchase of automated donation mixers appears unjustifiable.
2. **ANTI-OAGULANT**

ACD (pH 4.9), CPD (pH 5.7), sodium citrate (pH 8.4) and heparin result in a fresh plasma pH of 7.1, 7.4, 7.6 and 7.5 approximately.

Total citrate concentration is 113mM in ACD and 105mM in CPD. Laboratories studies (Vermeer 1976a, Working Party 1978) suggest that fresh CPD plasma contains more factor VIII than ACD plasma but that this advantage is lost if plasma preparation is delayed. In practice early workers found no difference between ACD or CPD (Goldstein 1964, Pool 1967, Slichter 1976) but more recent work, largely from the UK, shows CPD to be better than ACD, possibly even for plasma frozen after overnight holding of blood (Mooreside 1969, Schanberge 1972, Working Party 1978, Smith 1979a, Wensley 1980, Smith 1981, Lane 1981, Prowse 1981, Robinson 1983). By comparing results obtained with trisodium citrate apheresis plasma, Smith (1981) suggests this is due to pH differences rather than citrate concentration differences, although Pool (1967) shows both parameters may affect cryoprecipitate yield when excess ACD is added to plasma. Weiss (1965 and references therein) similarly showed excess citrate affects factor VIII stability in plasma but, it is interesting to note that Zucker (1982) found higher factor VIII levels in citrate plasma than in plasma anticoagulated by treatment with barium sulphate. Bayer (1980) has shown that use of trisodium citrate for plasmapheresis yields plasma with good factor VIII levels (0.85 u/ml) but it should be borne in mind that both for plasmapheresis and normal donation it may be possible to get as good results with lower citrate concentrations than are found in ACD or CPD, reduction of citrate levels by one-third having no adverse effects on red cell viability (Mishler 1978). However, this may reduce the current margin of safety in preventing coagulation during donation and storage. Mustard (1957, 1958) found there was an optimal concentration of citrate for factor VIII in plasma, too low a concentration giving greater lability. Recent data from Robinson (1983) suggests some benefit from reducing citrate from current levels. Use of adenine supplements in ACD or CPD has no adverse effect on plasma factor VIII (Mooreside 1969). Factor VIII in oxalated or EDTA blood is more labile than in citrated blood (Spaet, 1955, Mustard 1958).

RECOMMENDATION 2

1. Of the standard anticoagulants CPD is the one of choice.

2. Use of heparin or lower citrate anticoagulants may have possible benefits.

3. CENTRIFUGATION AND CELLULAR CONTAMINATION

Inadequate centrifugation resulting in cellular contamination of plasma may lead to problems in filtering blood products and for this reason Swedish plasma is spun twice to ensure a platelet count of < 15 x 10^7/1 (Council of Europe 1980). Prior to fractionation cellular contamination has no apparent effect on plasma factor VIII in fresh or stored blood (Preston 1967, Burk 1975, Slichter 1976, Pepper 1978, Counts 1979) although hard spinning to remove platelets may result in loss of up to 20% factor VIII due to cellular binding of the protein (Stibbe 1972). Mustard (1957) reported factor VIII to be more stable in thrombocytopenic blood but this has not been confirmed. In practical terms, centrifugation between 18,600 and 62,200g min giving platelet counts in plasma between 35 and 10 x 10^7/1 has no effect on the subsequent yield of factor VIII in cryoprecipitate, which is similarly unaffected by recentrifugation of plasma at 30,000g min (Working Party 1978, Lau 1983). Plasmapheresis yields plasma which may have a higher than normal platelet count (up to 50,000) but experience with such plasma shows no problems in terms of factor VIII yields, filterability or solubility of such material (Smith 1981, Robinson 1983, SBTS 1983) although very high platelet contamination is known to affect the filtration characteristics of factor VIII products (Smith 1977).

RECOMMENDATION 3

Centrifugation that results in plasma with a platelet count of < 30 x 10^7/1 is apparently adequate.

4. TEMPERATURE AND TIME FROM DONATION TO FREEZING

hours, over 4 hours, over 6 to 8 hours, over 18 to 24 hours, although such differences are not always significant (Vermeer 1976b, Pepper 1977, Smith 1978, Smith 1979b, Prowse 1981, Kellner 1982, SBTS 1983) and may not be reflected in the factor VIII yield in cryoprecipitate (Working Party 1978) or concentrate (Smith 1979b), but local results suggest they are (SBTS 1983). A report of no loss of factor VIII in blood stored 8 hours at 22°C (Sohmer 1982) should be regarded as unusual.

Above 30°C plasma factor VIII is more labile and losses of up to 40% within 2 hours are reported (Perkins 1962, Stibbe 1972, Vermeer 1976b) and a few studies purport such lability at 22°C (Rock 1980b) or even 4°C (Pepper 1978).

Vermeer has reported (1976a) that in blood cooled below 8°C factor VIII is lost into the red cell layer during centrifugation due to cryoprecipitation. This has been confirmed for factor VIII:C (Smith 1981, SBTS 1983 and factor VIII-related antigen, SBTS 1983 despite Hordow 1982). In practice, due to its thermal mass, blood is difficult to cool to this temperature, in bulk, in under four hours (Pick 1971, Gunson 1976, Rock 1979a) and loss of factor VIII by cryoprecipitation probably only occurs to a noticeable extent, if at all, in blood stored at 4°C for more than 6 hours (Pepper 1978, SBTS 1983). After 18 hours storage at 4°C small volumes of blood give apparent plasma VIII:C levels of 40% of that in fresh plasma. The true VIII:C level in such blood is 65%, the difference being due to cryoprecipitation which may be avoided by storage at 10°C or by rewarming at 37°C for 1 hour (SBTS 1983). In practice, recent local experience suggests that this effect is not seen during routine production of plasma from blood stored at 4°C and processed within 18 hours of donation (SBTS 1983).


RECOMMENDATION 4

1. Blood should be cooled after donation and the plasma separated and frozen as quickly as possible.

2. If delay in processing is unavoidable non-chelating anticoagulants such as heparin would seem attractive if they are compatible with the other products and processes of blood transfusion.
5. **FREEZING RATE**

Various studies suggest an inevitable loss of about 15% of factor VIII during freezing (Preston 1967, Verstraete 1969) but that freezing of small volumes of plasma may reduce this loss (Britten 1966, Kasper 1975, Rock 1977). The overall impression from the literature is that provided plasma is frozen to below -20°C within 1 to 2 hours, faster freezing provides no additional benefit. Thus placement of fresh plasma packs in liquid nitrogen, ethanol-cardice, -80°C or -60°C freezers makes little difference, but that simple placement in a -30°C or -20°C freezer results in increased (10%) loss of factor VIII, complete freezing under these conditions taking 6 hours or more (Preston 1967, Kasper 1975, Vermeer 1976a, Slichter 1975, Rock 1979a). Recent laboratory studies demonstrate that freezing in ethanol-cardice, taking about 15 minutes, results in better recovery of factor VIII than freezing in a -40°C or -70°C freezer, taking 4 hours or more; but differences are of the order of 10% or less (Fiets 1982, SBTS 1983). At the practical scale, Smith (1983) reports no difference in factor VIII content of plasma frozen as single units in a Pritchard blast freezer, as 5 litre pools in a Grant freezer or IPP (wedge) packs in a cooled plate freezer. It should be remembered the chosen freezer should have a suitable throughput capacity as well as an adequate freezing rate.

**RECOMMENDATION 5**

1. A freezer should ensure the plasma mass is frozen to below -25°C in less than 2 hours.

2. In terms of throughput capacity and safety consideration the most suitable items would appear to be mechanical blast freezers or liquid nitrogen/vapour equipment.

6. **FROZEN TEMPERATURE AND STORAGE TIME**

Measurements based on the theory of eutectics show phase changes occur in plasma at temperatures of -27°C, -40°C and possibly as low as -80°C and that storage at temperatures below these points might have advantages (Greaves 1968, Mackenzie 1982). In practice Anstall (1961), Perkins (1962) and Britten (1966) found only minor changes in factor VIII during storage at -30°C for up to 20 months. Subsequent workers have confirmed little or no loss of factor VIII in plasma frozen at -30°C or colder for periods up to 12 months (Preston 1967, Kasper 1975, Vermeer 1976a, Slichter 1976, Rock 1979a, Mooreside 1969, Orthner 1981, Koerner 1982, SBTS 1983, Snyder 1983). At -20°C results have been more variable, some showing a loss of factor VIII (Penick 1956, Anstall 1961, Kasper 1975, Slichter 1976, Rock 1979a) while others find no loss at this temperature (Rapaport 1960, Preston 1967, Koerner 1982, SBTS 1983). A study purporting to show losses of up to 70% of factor VIII during storage at -40°C must be regarded as unusual (Thomas 1982). Few of the studies above include data on actual temperatures recorded in refrigeration equipment.
Newman (1971) and local studies (SBTS 1983) suggest that storage of plasma in the frozen state for periods of longer than 3 to 6 months, while not affecting factor VIII, affects other proteins in plasma and can result in processing difficulties and high fibrinogen content during factor VIII concentrate production.

Partial thawing during frozen storage, eg due to mechanical failures, can result in dramatic loss of factor VIII of the order of 40% (Orthner 1981, Smith 1983, SBTS 1983) but this apparent loss may only be partially reflected in the yield of factor VIII in concentrate derived from such plasma (Smith 1983).

RECOMMENDATION 6

1. Plasma should be stored at -30°C or lower and processed as soon as possible.

2. Preventive maintenance of freezers should be planned and a safety margin (eg use of -40°C freezers) may also be desirable.
APPENDIX A (12/12/83)

Donor Procedure


Anticoagulant

1. Mikaelsson M E and Oswaldsson. (Thromb Haemost 50, 263, 1983) suggest the stability of factor VIII in heparin plasma is further improved by additional calcium.

Storage

1. Nilsson et al (Transfusion 23, 377-381, 1983) studied blood and plasma stored at 4°C and found little loss of factor VIII up to 6 hours, with an approximate 50% loss at 24 hours. The results suggest no "cryoprecipitation" of factor VIII under such conditions.
## Factor VIII in Feedstock

### (iu/ml)

#### Non-Scottish

<table>
<thead>
<tr>
<th>Value</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>0.8</td>
<td>Newman (1971) - American Red Cross: ACD</td>
</tr>
<tr>
<td>0.75</td>
<td>Brumelhuis (1976) - Dutch Red Cross: ACD</td>
</tr>
<tr>
<td>0.6</td>
<td>Guthrie (1976) - Australian CSL: FR4, ACD</td>
</tr>
<tr>
<td>0.74</td>
<td>Smith (1976) - Oxford BPL</td>
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<tr>
<td>0.39 - 0.95</td>
<td></td>
</tr>
<tr>
<td>0.84 FR6 in CPD</td>
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<tr>
<td>0.72 FR6 in ACD</td>
<td></td>
</tr>
<tr>
<td>0.77 FR18 in CPD</td>
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</tr>
<tr>
<td>0.70 FR18 in ACO</td>
<td></td>
</tr>
<tr>
<td>0.76 FR4</td>
<td>Lane (1981) - Elstree</td>
</tr>
<tr>
<td>0.52 FR18</td>
<td>Pepper (1978) - Leeds ACD</td>
</tr>
<tr>
<td>0.88 FR4</td>
<td>(4°C as blood or plasma)</td>
</tr>
<tr>
<td>0.79 FR8</td>
<td>Smith (1978) - Oxford BTS</td>
</tr>
<tr>
<td>0.73 FR18</td>
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</tr>
<tr>
<td>0.49 FR</td>
<td>Whitman (1976)</td>
</tr>
<tr>
<td>0.47 Indate (&lt;7d)</td>
<td>Wickerhauser (1976) CPD</td>
</tr>
<tr>
<td>0.39 Outdate (21d)</td>
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<tr>
<td>0.45 Indate (4-6d)</td>
<td>Bayer (1980) - Sodium Citrate &quot;Pheresis&quot; Plasma</td>
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<tr>
<td>0.85 FR</td>
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#### Scottish

<table>
<thead>
<tr>
<th>Value</th>
<th>Source</th>
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</thead>
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<tr>
<td>0.84 in FR6 CPD</td>
<td>Prowse (1981) - data from 1979</td>
</tr>
<tr>
<td>0.76 in FR18 CPD</td>
<td>- no difference single vs 5 litre packs</td>
</tr>
<tr>
<td>0.60 in FR6 ACD</td>
<td>but Inverness FR2 (singles) gave 0.93</td>
</tr>
<tr>
<td>0.49 in FR18 ACD</td>
<td></td>
</tr>
<tr>
<td>~ 0.61 in FR6 CPD</td>
<td>recent PFC data (1981)</td>
</tr>
<tr>
<td>~ 0.4 - 0.5 in FR18 CPD</td>
<td>Edinburgh QA 1982 (sa-ble pre-freeze)</td>
</tr>
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<td>0.75 in FR6 CPD</td>
<td>Glasgow QA 1981 (sample random frozen packs)</td>
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<tr>
<td>0.61 in FR6 CPD</td>
<td>Inverness QA 1982</td>
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<tr>
<td>0.35 - 1.5 in FR CPD</td>
<td></td>
</tr>
<tr>
<td>0.76 in FR6 CPD pool</td>
<td>Edinburgh CA 1983 (sa-ble pre-freeze)</td>
</tr>
<tr>
<td>0.65 in FR18 CPD pool</td>
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</tr>
<tr>
<td>0.85 in FR6 CPD single donation</td>
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<tr>
<td>0.53 in FR18 CPD single donation</td>
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<tr>
<td>~ 0.6 in FR6 CPD</td>
<td>PFC Data (1982)</td>
</tr>
<tr>
<td>~ 0.5 in FR18 CPD</td>
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</tr>
</tbody>
</table>
REFERENCES


COUNCIL OF EUROPE (1980) - Preparation and use of coagulation factors VIII and IX for transfusion.


GUNSON H (1976) - ibid


EBTS (1974) - unpublished observations from Edinburgh B T S.

STTS (1983) - unpublished observations from PFC and Scottish Regional Centres.