NATIONAL BLOOD TRANSFUSION SERVICE

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Your Ref.
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22nd June, 1977

Dr. R. Lane,
Consultant Haematologist,
National Blood Transfusion Service,
Regional Transfusion Centre,
Crescent Drive,
Brentwood,
ESSEX.

Dear Dr. Lane,

I have been trying to contact you by telephone today but I have been unsuccessful. However, I did not wish to delay sending you the report on the Cryoprecipitate. Ethel Bidwell as you know is also away and so I have put together the material we had available because I felt that we ought to present this at the July meeting of the Regional Transfusion Directors', since the next meeting after that meeting is not until October.

I realise that it is not an awfully satisfactory way of going about things and we should have had a discussion prior to submitting the report to the meeting, but I have had to send it to Dr. Maycock for circulation, because the agenda is to go out early next week.

I have spoken to Dr. Wensley and he has agreed, and I hope you will be agreeable also to having a look through this report and if there is anything with which you violently disagree or which you feel ought to have been included which has not been, a short addendum could be prepared to the report and circulated later. Alternatively you may wish to enlarge on some aspects at the Regional Transfusion Directors' meeting itself, and Dr. Maycock will be sending you an invitation to come to the meeting to discuss this item. He has agreed that it should be put on the first item following lunch and I hope you will be free to come for lunch on Wednesday 6th July at the Department of Health and Social Security at 1.00p.m. Alternatively if you would like to discuss any aspects of this report with me I would be very happy to come over to Elstree one day next week to do this.

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I hope you will be agreeable to these arrangements and I apologise for the rush, which was caused by the fact that we only completed the experimental work last week.

With kind regards.

Yours sincerely,

GRO-C

H.H. Gunson M.D.

Enc.

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REGIONAL TRANSFUSION DIRECTORS' COMMITTEE

REPORT OF THE WORKING PARTY ON THE QUALITY OF CRYOPRECIPITATES IN ENGLAND AND WALES

H.H. GUNSON (CONVENOR)
E. BIDWELL
R.S. LANE
R.T. WENSLEY
T. SNAPE (Co-opted)

JUNE 1977

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SUMMARY AND RECOMMENDATIONS

1. The Working Party have carried out investigations into seven variables in the preparation of cryoprecipitates as follows:

   (i) Selection of donations.
   (ii) Choice of anticoagulant - CPD versus ACD.
   (iii) Age of starting plasma - less than 4 hours, 4-6 hours, 18 hours.
   (iv) Age of starting plasma combined with choice of anticoagulant.
   (v) Conditions of centrifugation.
   (vi) Conditions of freezing the plasma.
   (vii) Conditions of thawing times of the plasma combined with choice of anticoagulant and age of starting plasma.

2. Certain limitations of experimental design were necessarily imposed and in many experiments the variability of Factor VIII activity in pools of two group O and two group A cryoprecipitates meant that differences in Factor VIII activity less than 35% could not be confirmed.

3. No evidence was found to suggest that the age of the starting plasma, conditions of centrifugation or the conditions of freezing the plasma exerted a significant effect on the Factor VIII activity in the product.

4. Experiments on the selection of donations were inconclusive, but other studies showed that there was a good correlation between the Factor VIII activity in the plasma before freezing and that found in cryoprecipitate. As a general routine, however, screening of donors does not seem to be practical.

5. When the plasma used for cryoprecipitates was less than four hours old and possibly up to six hours old, CPD appeared to be the anticoagulant of choice. The differences in Factor VIII activity between cryoprecipitat prepared from ACD and CPD plasma which was 18 hours old was not significant

6. Rapid thawing of plasma appears to be advantageous although the process leads to considerable variation in Factor VIII activity in small pools.

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1. INTRODUCTION

The working party was convened at the RTD meeting on 21st July 1976 with the following terms of reference:

"To examine the quality of cryoprecipitates produced at Regional Transfusion Centres and the factors affecting it."

The membership comprised:

Dr. E. Bidwell - Plasma Fractionation Laboratory, Oxford
Dr. H. H. Gunson - R.T.C. Oxford (Convenor)
Dr. R. S. Lane - R.T.C. Brentwood
Dr. R.T. Wensley - R.T.C. Manchester

Mr. T. Snape, PFL, Oxford was co-opted

The working party has met formally on one occasion only, on 22nd November 1976, to consider the preliminary report and to plan further investigations.

The preliminary report was presented at the RTD meeting on 8th December 1976. In this report, the results of the survey of Factor VIII activity in randomly selected cryoprecipitates sent to the PFL from each RTC, was reviewed. It was shown that there was a significant difference in the Factor VIII levels in cryoprecipitate produced at different RTCs. In part this was due to variation in the initial volumes of plasma used for cryoprecipitation. It was apprent, however, that there were considerable variations with respect to the preparation of cryoprecipitates. It was agreed that visits should be made to each RTC in England and Wales to study the variables involved and that certain RTCs should be asked to provide cryoprecipitates for the investigation of the different factors involved.

Visits to the RTCs by members of the working party were carried out between November 1976 and March 1977. A survey of the findings is contained in Section 2 of this report.

As a result of these visits co-operation was obtained for experiments to study the following variables which are described in Section 3.

Selection of donors
Choice of anticoagulant - ACD versus CPD.
Age of starting plasma

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Age of starting plasma combined with choice of anticoagulant

Conditions of centrifugation

Conditions of freezing the plasma

Conditions of thawing: slow thaw versus rapid thaw, combined with choice of anticoagulant and age of starting plasma.

Although not part of the brief of this working party, certain observations were made during the visits to the RTC's on the pooling of plasma into five-litre packs for the preparation of Factor VIII concentrate. A summary of the observations can be found in the addendum to this report

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2. A SURVEY OF CRYOPRECIPITATE PRODUCTION AT THE REGIONAL TRANSFUSION CENTRES IN ENGLAND AND WALES

In order to analyze the problems involved and the methods adopted for the preparation of cryoprecipitates in each region a questionnaire was completed at the time of the visit during discussions with the Director and the Senior Technical Staff involved with the process. A summary of the findings is given below.

1. Number of donations used to prepare cryoprecipitate

It can be seen from Table I that in England and Wales, as a whole, cryoprecipitates make a major contribution to the supply of Factor VIII with more than 250,000 donations committed annually to its production, i.e., approximately 15 per cent of the total number of donations of blood collected each year. The majority of Directors considered that this annual rate of production would have to continue for the foreseeable future, since, in only four regions, were the future estimates lower than at present and then only by a small margin.

It is evident, however, that there is variable cryoprecipitate production in individual Regional Transfusion Centres in terms of the proportion of the total donations used. In one-half of the Regions 8 - 15 per cent of the donations are used for cryoprecipitate preparation but the limits vary widely from over 31 per cent in Edgware to 0.7 per cent in Oxford, and special factors within the regions are responsible for this.

Most cryoprecipitate preparations are used in recognized Haemophilia Centres which usually are situated in the same city as the RTC, although, in certain regions, e.g., Sheffield, the material has to be transported considerable distances.

2. Collection of Blood for cryoprecipitate preparation

(i) Selection of sessions

Geographical considerations in the regions largely determine the sessions from which blood is used for the preparation of cryoprecipitate. It can be seen from Table II that the blood is collected from sessions which are most frequently five to 25 miles from the RTC. Only one RTC (Manchester) collects an appreciable proportion of the total donations used for cryoprecipitate preparation within the RTC itself, and two RTCs (Cambridge and Lancaster) collect a significant proportion from sessions greater than 50 miles distant.

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These questions were asked to obtain a general assessment of Regional differences should it be found subsequently that the age of the starting plasma was important. However, it became clear that using the criterion of distance of the sessions from the Centre may give a misleading picture. In the large urban conurbations, particularly in London and the West Midlands, traffic congestion is a much more important factor than distance. This is well illustrated at the Brentwood RTC where travelling time from the Centre to each of the regional boundaries is similar, whereas, westwards, into London, was less than 20 miles and Harwich was in excess of 50 miles. On the other hand, certain Centres in largely rural areas have motorways bisecting the region allowing relatively easy transport of blood from the sessions to the RTC, e.g., Lancaster.

(ii) Selection of donors

Donors are not selected with respect to age or sex at any of the RTCs, and in only three Centres, is there any selection with respect to blood group. In the Leeds and Liverpool regions, cryoprecipitate is prepared largely from Group O and Group A donations, while in Manchester, a proportion of Group O donations are used for platelet preparations so that there is an excess of Group A blood used for cryoprecipitates.

In several regions there is a relatively high immigrant population, but, with respect to the donor population, it is generally considered that there is no unusual racial proportion.

(iii) Selection of donations

In six of the 15 Centres, notice is taken of the time of donation and difficulties with the venepuncture. Donations which are difficult to obtain or do not flow freely are not used for the preparation of cryoprecipitates, although, in one region, plasma from such donations was included in five litre packs for fractionation. In general, times of donations are not recorded and the rejection of a unit of blood is dependent on the decision of the sessional Medical Officer or the mobile team staff. In the Oxford region a survey of the time taken for donation was carried out, and 50% of donations were found to take longer than ~~ten minutes~~.

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This value is at variance with most estimates, which suggest a much higher percentage completed within ten minutes.

ACD is the anticoagulant in most common use for donations from which cryoprecipitate is prepared, but CPD is used at the RTCs in Edgware and Birmingham. The use of CPD plasma had been discontinued at Southampton because of difficulties with harvesting the cryoprecipitate (see Section 3ii(b)). At the Bristol RTC, cryoprecipitates for paediatric use are selected from donations in which the Factor VIII activity in the staring plasma exceeds 70%.

(iv) Transport of units of blood to the Regional Transfusion Centre

In only three regions, (Tooting, Oxford and Bristol), blood is transported from mobile team sessions to the Centre, without cooling, for cryoprecipitation on the same day. In two Centres, (Edgware and Manchester), donations collected in the RTC are not cooled prior to cryoprecipitate preparation. Investigations in the Oxford Region have shown that, without cooling, there was a seasonal variation in the temperature of the plasma by the time separation was carried out, but this was principally in the range of 10 - 25°C.

In other regions, cooling often commenced on the blood collection sessions and the units of blood are transported to the RTC in refrigerated compartments at 4°C or in insulated boxes with ice inserts. Estimates of the temperature of the plasma at the time of separation, when blood donations were handled in this manner, are not available.

In two regions, cryoprecipitate is prepared from 18 hour-old plasma having been placed in a 4°C refrigerator overnight. It can be assumed that the temperature of the plasma at the time of separation is also 4°C. In other RTCs, the units of blood for cryoprecipitate are usually processed without a significant storage period prior to centrifugation.

3. Preparative work within the Regional Transfusion Centre

(i) Staffing

In general, the direct supervision of the preparation of cryoprecipitates is carried out in all RTCs by either a Senior

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Chief Technician, or more commonly, a Chief Technician or Scientific Officer. In the majority of Regional Transfusion Centres, other staff involved in cryoprecipitate preparation are technicians and/or junior technicians, although, in three Centres, the staff are predominently ancillary supervised by qualified technicians.

(ii) Centrifugation

(a) Initial centrifugation of blood packs

In all except one Regional Transfusion Centre, (Edgware), MSE 6L or Coolspin centrifuges are used. At N.W. Thames Regional Transfusion Centre, Damon and Sorval centrifuges are used in addition to MSE.

It can be seen from Table III that centrifugation conditions vary. Most commonly (nine RTCs), a six place swing-out head operating at the maximum speed of 2,500 r.p.m. (2075g) was employed for periods varying from 15 to 30 minutes at full speed. The shortest period of centrifugation at this speed was found at Tooting RTC, i.e., nine minutes using a six place angle head. Angle heads either 4-place or 6-place were used at six RTCs at higher speeds, but, for correspondingly shorter times, varying from four minutes at 3850g. at Brentwood to 20 minutes at 3500g. at Newcastle. The highest centrifugal force employed was in the Sorval centrifuge at Edgware, i.e., 6150g. for five minutes.

At three RTCs, Edgware, Tooting and Birmingham the preparation of cryoprecipitates was combined with the preparation of platelet concentrates. Estimates of the platelet count in the plasma used to prepare cryoprecipitate have not been determined except at three RTCs, (Edgware, Tooting and Oxford). In each instance, it has been shown that the platelet counts on the separated plasma were in the range of 20 - 60 x 10^9 per litre, and at Oxford RTC, it has been shown that the longer slower spin in the swing out head consistently produced lower platelet counts than the faster quicker spins in angle heads.

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(b) Centrifugation to harvest cryoprecipitate (Table III)

Usually, the same centrifuges are used for the harvesting of cryoprecipitate with similar speeds as the initial spin, although, in some instances, a shorter period of centrifugation is employed.

The longest period of centrifugation is used at the Oxford Regional Transfusion Centre (1200g. for 30 minutes) and this is caused by the method employed at that Centre. The plasma from two donations is pooled in an MRC blood bottle and frozen at $-30^{\circ}C$. After thawing at $4^{\circ}C$ for a period of 18 - 20 hours, the cryoprecipitate is harvested.

At the Southampton RTC a second centrifugation is not carried out. Experiments at that Centre suggest that improved yield of Factor VIII and the procedure of draining the plasma from the cryoprecipitate is clearly time-saving. However, it was stated that the finer precipitate obtained from CPD plasma could not be harvested by this technique.

(iii) Separation of centrifuged plasma

With the exception of Oxford Regional Transfusion Centre, where pigtail packs are used, Fenwall double or triple packs are used for donations for cryoprecipitate preparation. Also, Fenwall extractors are used in all Centres except Oxford and the time taken to express the plasma is in the order of 1 - $1\frac{1}{2}$ minutes. Locally made extractors are in use in the Oxford RTC and the time taken to remove the plasma from a unit of centrifuged blood is approximately 20 seconds.

(iv) Handling Time

From the foregoing, it is apparent that factors exist at RTCs which influence the age of plasma from which cryoprecipitate is prepared. Three different periods of time have been defined from the time of donation to the freezing of the plasma, i.e., less than four hours, four to six hours and 18 hours. Table IV shows the percentage of donations used for cryoprecipitate preparation at each RTC which fall into the above periods. It can be seen that the two extremes are all the donation of plasma frozen within four hours (Sheffield, where a mobile laboratory is used at the sessions, Manchester and Southampton) and all donations of plasma frozen after 18 hours (Newcastle).

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Overall 40% of cryoprecipitates are prepared from plasma frozen in less than four hours, 50% in 4 - 6 hours and 10% after 18 hours.

(v) Conditions for Freezing and Thawing Plasma

Ethanol/CO_2 is the commonest coolant used for freezing the plasma for cryoprecipitation and in general, it can be seen from Table V the bath temperature was in the range of -60 to -70°C, although in six Regional Transfusion Centres, this was based on assumption rather than by measurement. Agitation was achieved by the release of CO_2 bubbles from the liquid. The period of freezing was variable, from 7 to 30 minutes.

Experiments carried out by Mr. G.W.R. Dike of the Plasma Fractionation Laboratory Oxford have shown that in Ethanol/CO_2, the centre of the plasma in the pack reaches -40°C after an average of 15 minutes.

At the Birmingham RTC, a liquid nitrogen plate freezer regulated to give plate temperatures of -60°C to -70°C is used and in Oxford the plasma is frozen in glass bottles in a -30°C freezing room.

At the Southampton RTC, aluminium formers were placed on each of the flat faces of the bag containing separated plasma during the freezing process. This allowed the pack to freeze with a uniform depth of plasma in the pack instead of the "pear" shape that the container normally assumes during freezing.

Usually, the tahwing process is commenced shortly after freezing and commonly this is carried out by hanging the bags of cells and separated plasma in a cold room at +4°C with adequate air circulation. The time of thawing is usually overnight but varies from 18 to 24 hours depending on the time of the day the plasma is frozen.

At the Newcastle RTC, a rapid thaw procedure is used in a waterbath containing distilled water maintained at +8°C. Packs of frozen plasma in an outer covering pack were placed in the waterbath for a period of approximately 2½ hours. Experiments on rapid thaw are in progress at one RTC.

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(vi) Storage of cryoprecipitate

Most commonly cryoprecipitates are stored at -30°C to -40°C while awaiting delivery to the hospitals, although, at two RTCs, the storage temperature was -25°C. It was noted that, at some RTCs, the monitoring of the temperature of the freezers used for cryoprecipitate was not supervised by the staff concerned with their preparation and in certain instances these staff were unsure of the conditions under which the material is stored.

Expiry dates vary from three months to six months, although it was commonly stated that manufacture and issues are arranged so that a turnover took place over a short period. Measures to ensure proper turnover of cryoprecipitates were in operation at each RTC.

(vii) Quality Control

Assessment of Factor VIII activity in cryoprecipitates is carried out in six RTCs, although the number of cryoprecipitates tested compared with the number prepared varied from 1% to 3%. Standards for assay vary as did the assay techniques. The removal of the cryoprecipitate from the pack was, in all instances, performed in a manner similar to that used when preparing a dose.

In the remaining Regional Transfusion Centres, the situation is very variable. Quality control of Factor VIII activity is carried out by the local Haemophilia Centre, but communications between the Haemophilia Centre and the RTC varied from close co-operation to nil.

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3. Investigation of some of the variables involved in the preparation of cryoprecipitates

It was apparent from the visits to the RTCs that many variables existed in the preparation of cryoprecipitates. With the co-operation of the Directors of several Centres, certain of the variables have been examined. Since production of cryoprecipitates was geared to clinical demand, experiments were designed so that they caused the least disruption of the normal work of the department in the RTC. This policy has placed some limitation on experimental design but it should be stressed that the requests made by the Working Party inevitably led to a considerable amount of extra work for staff in hard-pressed departments in the RTCs.

In general, when studying a given variable, the RTC concerned was asked to send eight group O and eight group A cryoprecipitates prepared in one manner and a similar number prepared differently for comparison to the PFL, Oxford, for Factor VIII assay. In order that comparisons be meaningful, requests were made to keep other variables constant, e.g. if the variable was CPD plasma versus ACD plasma, then the 16 cryoprecipitates prepared from each type of plasma should have been from the same age of plasma, frozen under identical conditions, the centrifugation should have been constant, the initial and residual plasma volumes should have been closely controlled and storage conditions of the cryoprecipitates prior to transport to the PFL should have been the same.

When the 16 cryoprecipitates of each type were received at the PFL, four pools, comprising two group A and two group O cryoprecipitates, were prepared for each type, as follows: the volume of each cryoprecipitate was measured and recorded; each pack was rinsed out with 5 ml saline which was added to the cryoprecipitate and the four cryoprecipitates pooled and the pool volume made up to 200 ml using 150 mM saline. Occasionally cryoprecipitates of volume greater than 50 ml were encountered - if this happened the pool was made up to the smallest suitable volume and allowance was made for the dilution in the calculati that followed. If one cryoprecipitate of a pool leaked, or was damaged in some way, the pool was limited to one group O and one group A cryoprecipitate to rule out, as far as possible, variation in factor VIII activity due to blood group.

Cryoprecipitates were thawed and the pools prepared immediately before assay. The design of each exercise was such that one cryoprecipitate pool from each of the types being studied on any one occasion was assayed simultaneously by two different operators. Thus, in the exercise described in section 3.4 (Table IX), each block consisted of four operators assaying two of the four pools prepared. The scheme is illustrated below:

	CPD, < 4 hours	ACD, < 4 hours	CPD, 18 hours	ACD, 18 hours
Pool numbers	1 2 3 4	5 6 7 8	9 10 11 12	13 14 15 16

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Block	Operator 1	Operator 2	Operator 3	Operator 4
1	1 + 5	9 + 13	5 + 9	1 + 13
2	10 + 14	2 + 6	2 + 14	6 + 10
3	3 + 15	11 + 7	7 + 15	3 + 11
4	8 + 12	16 + 4	12 + 4	16 + 8

Similar schemes were adopted for the other exercises. In this way, an appropriate number of operators could be employed so that comparison of the different cryoprecipitate types could be made simultaneously whilst the restricted randomisation of the pools between the operators meant that between operator variation could be eliminated as far as possible.

A two-stage factor VIII assay was employed throughout. Samples were assayed in a set of the form S T_1 T_2 T_2 T_1 S against the 5th British Plasma Standard for factor VIII. An initial 1 in 4 dilution in haemophilic plasma was made for each cryoprecipitate pool. The plasma standard and the dilutions in haemophilic plasma of the test samples were all adsorbed with aluminium hydroxide suspension (1.3% w/v). Only assays which satisfied the usual criteria of validity were included in the final analysis. For each exercise after between operator variation had been ruled out, a combined log potency was calculated for each cryoprecipitate pool. Although Tables VI to XVI present the potencies as 'iu factor VIII per cryoprecipitate', the necessary t-tests and analyses of variance were carried out on the log potency estimates and the mean values shown were always obtained as the arithmetic mean of the log potency estimates. Similarly, the best expression of the variation between the observations on any one type of cryoprecipitate is the standard deviation of the log potency estimates expressed in antilogarithmic form as a percentage.

3.1 Selection of Donors

At the Bristol RTC, donations used to prepare cryoprecipitates for paediatric use are selected by carrying out Factor VIII assays on the starting plasma. The initial selection comprises those plasmas which have 70% or greater Factor VIII activity. Based on the assumption that the yield of Factor VIII in the cryoprecipitate is 40%, the final selection is made by calculation from the starting plasma volume to give a Factor VIII activity of at least 80 units per pack.

It can be seen from Table VI that the difference between the geometric mean potencies for the selected and unselected cryoprecipitates is highly significant ($t = -6.15$, $0.001 < P < 0.01$). However, inspection of the Factor VIII levels in the unselected routine cryoprecipitates is unexpectedly low, and much less than that obtained for the Bristol RTC in the preliminary survey previously reported (74.6 iu to 96.0 iu; mean 86.6 iu per pack). The significant difference between the two types of cryoprecipitate is therefore more likely to be due to the unusually low yield of factor VIII of the unselected cryoprecipitates rather than a higher yield in those selected for paediatric use.

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3.2 Choice of anticoagulant, ACD versus CPD

At the Birmingham RTC, 90 per cent of the plasma routinely used for the preparation of cryoprecipitates is anticoagulated with CPD. The plasma is between four and six hours old at the time of freezing. Comparison of the Factor VIII activity of cryoprecipitates supplied by the Birmingham RTC derived from ACD and CPD plasma is shown in Table VII. The considerable variability of potency of the cryoprecipitates prepared from the ACD plasma reduced the power of this comparison. However, the difference between the geometric mean potencies for ACD and CPD derived cryoprecipitates was almost significant at the 5% level ($t = -2.00$, $0.05 < P < 0.1$).

3.3 Age of starting plasma

Three criteria were set with respect to the age of the plasma, viz. freezing within four hours of the collection of the blood, within four to six hours, and overnight, assumed to be 18 hours. Three RTCs, Liverpool, Brentwood and Manchester, co-operated with this experiment and the results of Factor VIII assay of the resultant cryoprecipitates are shown in Table VIII.

Since no single Transfusion Centre prepared as routine cryoprecipitates from all three types of plasma, a more complex design had to be adopted for this multiple comparison. The corrected treatment means were assigned by calculating an overall mean and adding or subtracting a correction term based on the interaction between treatments and blocks. Analysis of variance of the resulting balanced incomplete block design suggested no significant difference between the mean potencies of the three types of cryoprecipitate.

3.4 Age of starting plasma combined with choice of anticoagulant

Manchester RTC collected donations of blood into plasma anticoagulated with CPD and ACD and prepared cryoprecipitates from both types of plasma in less than four hours and a second set from plasma stored at 4°C for 18 hours after donation.

It was unfortunate in this detailed experiment that split plastic packs curtailed the pools of cryoprecipitate to one group O and one group A in four of the pools (Table IX). An analysis of variance performed on the results suggested that the differences between the geometric mean potencies were not significant overall. However, because of the uneven distribution of variance between the four groups, possibly resulting from the differences in pool size (S.D. for the ACD/4 hour cryoprecipitates was three times that for CPD/18 hour cryoprecipitates), it seemed likely that a paired t-test for comparisons of particular interest might be more helpful.

Such comparisons, in the context of the experiments carried out, yielded the following information:

(i) A significantly higher Factor VIII activity was observed in the cryoprecipitate prepared from CPD plasma less than four hours old, compared with that after storage of the plasma for 18 hours ($0.02 < P < 0.05$).

(ii) Significantly higher Factor VIII levels were obtained in cryoprecipitate derived from CPD plasma in less than four hours compared with those for ACD plasma after 18 hours' storage ($0.02 < P < 0.05$).

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(iii) No significant difference was observed between the Factor VIII activity of cryoprecipitates prepared from ACD and CPD plasma 18 hours after blood collection.

3.5 Conditions of centrifugation

Conditions of centrifugation varied considerably between the various RTCs, both with respect to rotor speeds and time of centrifugation. Three experiments were undertaken to determine whether the different centrifugation conditions exerted a significant effect on the Factor VIII activity in cryoprecipitates.

3.5.1 Comparison of different rotor speeds

At Edgware RTC, three types of centrifuge are used for the initial centrifugation of the plasma. These are the Mistral 6L with a six-place swing-out head at 2500 rpm (2075 x g) for 15 minutes, the Damon centrifuge with a six-place swing-out head at 4200 rpm (4850 x g) for seven minutes, and the Sorval centrifuge using a four-place swing-out head at 5000 rpm (6450 x g) for five minutes.

From Table X it can be seen that there was no significant difference between the mean Factor VIII activities of the cryoprecipitates prepared from plasma centrifuged under these three conditions.

3.5.2 Comparison after different times of centrifugation

The most common condition for centrifugation of the starting plasma was in a Mistral 6L centrifuge at 2500 rpm (2075 x g). However, the times for centrifugation varied between the Centres from nine to 30 minutes (Table III). At the Oxford RTC experiments were carried out in an attempt to demonstrate differences in the Factor VIII activity resulting from the extremes of centrifugation at 2075 x g.

The results (Table XI) clearly indicate that differences between the two group means could be explained in terms of the variation within the groups.

3.5.3 Double centrifugation

In order to determine whether a second centrifugation of the starting plasma would have a significant effect on Factor VIII activity in the resulting cryoprecipitate, the following experiment was carried out at the Oxford RTC.

Six group O and six group A donations were collected and within four hours each donation was centrifuged in a Mistral 6L centrifuge with a six-place angle head at 4000 rpm (3850 x g) for ten minutes. Six pools (Table XII) were made by combining exactly 200g of group O and 200g of group A plasma. Each of the six pools was then split into two halves; one was immediately frozen in ethanol/CO_2 to prepare cryoprecipitate. The other half was re-spun under the same conditions, the supernatant plasma removed and frozen to prepare cryoprecipitate. An estimated 5-10 ml (2½% to 5%) was lost on each of these transfers.

The difference between the mean values for the two types of cryoprecipitate was not significant at the 10% level.

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3.6 Conditions of freezing the plasma

Each RTC froze plasma at -60°C to -70°C. The only variable was the time the plasma was allowed to remain in contact with the coolant, which varied from seven to 30 minutes. Since experiments had shown that the centre of a pack reached -40°C after 15 minutes under these conditions (see section 2), Sheffield RTC, who used the shortest time of seven minutes in their mobile laboratory, were asked to submit cryoprecipitates prepared from plasma which had been allowed to remain in ethanol/CO_2 for seven and 15 minutes respectively.

It is clear from Table XIII that the length of time that the starting plasma was in contact with the coolant did not significantly affect the Factor VIII activity of the resulting cryoprecipitate.

3.7 Comparison of thawing of plasma combined with choice of anticoagulant and age of starting plasma

The experiments in this section compared various parameters and were undertaken with the co-operation of the Newcastle and Oxford RTCs.

3.7.1 At Newcastle RTC a rapid thaw of the frozen plasma is a standard procedure. The cryoprecipitates are normally prepared from ACD plasma which is 18 hours old, but for the purpose of this investigation the staff at the RTC agreed to prepare cryoprecipitates for Factor VIII assay by

(i) their usual method, i.e. 18 hour plasma with a rapid thaw of 2½ hours at +8°C;

(ii) 18 hour plasma with a slow thaw at +4°C in a cold room overnight;

(iii) 4 hour plasma with a rapid thaw of 2½ hours at +8°C.

The results of the Factor VIII assays on the three types of plasma are shown in Table XIV. Analysis of variance shows a highly significant difference between the three types of cryoprecipitates ($P = 0.005$). Comparison of the individual group means by a paired t-test indicates that the mean Factor VIII activity for the slow-thaw cryoprecipitate was significantly lower than that for the two types of cryoprecipitate prepared by the "rapid-thaw" method ($P = 0.05$). The difference in Factor VIII activity between the means for the 18 hour/"rapid thaw" and the 4 hour/rapid thaw cryoprecipitates was not significant ($P = 0.10$).

3.7.2 At the Oxford RTC an attempt was made to confirm the advantages of "rapid thaw" versus "slow thaw" under experimental conditions which reduced the variability of Factor VIII levels in the donations of blood used for the experiment. 12 donations of 200g plasma, after centrifugation in a Mistral 6L centrifuge at 4000 rpm (3850 x g), were pooled in a large polythene pack. From this pack, 12 equal aliquots were prepared in separate packs. All the aliquots were placed in ethanol/CO_2 for 25 minutes, less than four hours after donation. Six packs were thawed overnight (about 18 hours) at 4°C in a cold room with air circulation and six were thawed in a waterbath at +8°C for 1½ hours.

The results of the Factor VIII assays on the six paired types of cryoprecipitates are shown in Table XV. The Factor VIII activity of the No. 2 cryoprecipitate in the "rapid thaw" group was clearly atypical and, omitting the results in this block, a paired t-test revealed a difference between the group means significant at better than 1% ($0.001 < P < 0.01$). 71/65

3.7.3 Since previous experiments (see sections 3.2 and 3.4) had suggested that under certain conditions CPD plasma gave advantages over ACD plasma it was decided to combine "slow" and "rapid" thaw with the two anticoagulants. The experiments were carried out at the Oxford RTC. 12 donations of blood taken into ACD and 12 taken into CPD were treated as described in section 3.7.2 above. The results of Factor VIII assays on the cryoprecipitates obtained are shown in Table XVI. Each plasma was frozen within four hours after collection of the blood donation.

Analysis of variance for the two main effects revealed no significant differences. Again, however, there was an uneven distribution of variance between the four groups and this suggested that more useful information might be obtained by using a paired t-test between the various groups. There was only one significant difference, that between ACD and CPD plasmas using the slow thaw method ($0.01 < P < 0.02$).

4. Discussion

Investigation of the methods for the preparation of cryoprecipitate in the RTCs in England and Wales indicated that there are many variations in the procedures employed. Some variables are unavoidable; thus, the age of the plasma prior to freezing is determined largely by either the geographical features of the region with respect to availability of donations being handled within four hours, four to six hours or overnight (18 hours). One region (Sheffield) overcomes these problems by the use of a mobile laboratory. Other factors, such as the choice of anticoagulant, centrifugation conditions, procedures for freezing and thawing and storage conditions, could be standardised if a significant advantage was apparent.

In the investigation of the variables involved in the preparation of cryoprecipitates the Working Party had to face the reality that limitations in experimentation were imposed by the fact that 15 per cent of donations are being converted into cryoprecipitates for clinical use. While RTCs realised that it was important to investigate the problem, resources were not available for detailed experimentation and the studies which were carried out were done only with considerable effort on the part of the RTCs concerned.

The principal factors which make the examination of Factor VIII activity of cryoprecipitates difficult with respect to variables in their preparation are:

(i) the variability of Factor VIII levels in individual donations.

(ii) the difference in activity between group O and group A cryoprecipitates which necessitates each pool being an equal mixture of the two groups.

(iii) a proportion of defective packs means that smaller pools must sometimes be included. This could, and on occasion (see section 3) did, result in high variability of Factor VIII assay.

(iv) while the study of a particular variable is being conducted it may be difficult to keep other variables constant. An example of this problem can be cited in the investigation of the Factor VIII levels with respect to the age of the plasma. Liverpool RTC agreed to send to the PFL, Oxford, for Factor VIII assay cryoprecipitates prepared from plasma which was less than four hours

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old and four to six hours old. 16 (eight group O and eight group A) cryoprecipitates were sent to the PFL from each group and the Factor VIII assay of pools of two group O and two group A cryoprecipitates of the two groups showed a significantly higher Factor VIII activity in the cryoprecipitate derived from the four to six hour plasma than from the less than four hour plasma. Further analysis revealed that the cryoprecipitates derived from the four to six hour plasma were contained in a residual plasma volume which averaged 39.0 ml per cryoprecipitate, while those obtained from plasma less than four hours old averaged 25.0 ml per cryoprecipitate. A good correlation existed between Factor VIII and cryoprecipitate volume ($r = 0.91$), suggesting that the cryoprecipitate volume was the over-riding factor. This might suggest that cryoprecipitate is lost when smaller residual plasma volumes are obtained but such a conclusion is not borne out by the results of the preliminary investigation reported previously, when the Manchester RTC recorded the overall smallest residual volume of cryoprecipitate per bag, yet attained Factor VIII levels in excess of average. The most likely explanation is that there was a systematic difference in the two groups of cryoprecipitate involving several factors.

In the experiments which were carried out with respect to the age of the starting plasma, the conditions of centrifugation and the conditions for freezing the plasma, no evidence was found to suggest that these factors exerted a significant effect on the Factor VIII activity in the resultant cryoprecipitates.

The experiments on the age of the starting plasma were limited by the fact that no RTC prepared plasma from each of the three categories. The statistical analysis of the complex design which had to be employed showed no significant differences. Comparisons might have been affected, however, by such factors as the volume of starting plasma which was probably not constant in the three Centres concerned. In the initial survey, reported previously, these volumes varied between 180 ml and 250 ml.

Slichter et al (1976) concluded that the Factor VIII activity in whole blood stored at $+4^{o}C$ declined steadily over 24 hours, and the yield of Factor VIII activity in cryoprecipitates stored for six hours was approximately 10 per cent less than for those prepared immediately after collection. The variability of the pools of cryoprecipitate tested in the present survey was such that a difference of over 30 per cent in the Factor VIII activity would have been necessary to be seen as significant (vide infra). It is clear that to demonstrate differences in the order of 10 per cent change in Factor VIII activity most closely controlled experiments will have to be carried out. It may well be that the labile portion of the Factor VIII in plasma is so short-lived that even four hours is too long to make an appreciable difference in the recovery of Factor VIII in cryoprecipitates.

Different centrifugation conditions had no effect on the Factor VIII activity of cryoprecipitates. The degree of centrifugation will affect the platelet concentration of the plasma prior to freezing. Several investigators, Burka et al, 1975 a, b, Slichter et al, 1976, have concluded that the platelet concentration, if below 25×10^{9}/litre, is not significant. The experiments in this study, particularly those involving the double spin, would seem to confirm this view. The presence of platelets at the concentration obtained on centrifugation for even the shortest time at the lowest speed (nine minutes at 2075 x g) did not reduce the Factor VIII activity in cryoprecipitates.

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The variation in the time for freezing at -60°C to -70°C was from seven minutes to 30 minutes. It has been shown that a temperature of -40°C was not reached in a single pack of plasma until 15 minutes after immersion at -60°C (see section 2). No significant difference in Factor VIII activity was found, however, in cryoprecipitates frozen for seven and 15 minutes. Obviously the holding time and temperature are not critical once the plasma has been frozen. This is not inconsistent with the observations of Vermeer et al (1976).

While the variables discussed above showed no significant differences, interesting observations were made with respect to the choice of anti-coagulant and the thawing process. Comparisons of the anticoagulants ACD and CPD were made in experiments 3.2, 3.4 and 3.7. In each instance differences were noted with respect to the choice of anticoagulant. These may be summarised as follows: when using plasma less than four hours old to prepare cryoprecipitates, CPD plasma gave significantly better yields of Factor VIII activity than ACD plasma. The advantage, although evident when using plasma between four and six hours old, seems to disappear with 18 hour old plasma. CPD plasma has a higher pH than ACD plasma, and Pool (1967) reported that the pH of the plasma influenced the yield of Factor VIII in cryoprecipitate and found that there was a 40 per cent loss if cryoprecipitates were prepared from acidified plasma. She found that there was a higher mean activity in cryoprecipitates prepared from CPD plasma compared with those from ACD, and this fact was confirmed by Shanberge et al (1970) for plasmas frozen shortly after collection. On the other hand, Graybeal et al (1969), Burka et al (1975) and Slichter et al (1976) concluded that there was no advantage in the use of CPD as an anticoagulant compared with ACD. Vermeer et al (1976) concluded that if plasma was stored then ACD was the anticoagulant of choice. The experiments conducted in the present study are consistent in that they showed significantly increased yields of Factor VIII in cryoprecipitate from plasma freshly collected and possibly up to six hours old only when CPD anticoagulant was used. Since 90% of cryoprecipitate is obtained from plasma within six hours after donation, this may be an important factor.

Advantages from the rapid thawing of the frozen plasma have been described by Slichter et al (1976) and Vermeer et al (1976) although this has not been confirmed by all workers (Kasper et al (1975)). The results of the experiments on thawing the frozen plasma in the present study proved interesting. There was no doubt that the Factor VIII activity in cryoprecipitates from the Newcastle RTC, where rapid thaw at +8°C of frozen plasma 18 hours old was routine, showed a significantly higher value than the same material subjected to a "slow" thaw. Preliminary results from the Manchester RTC (Wensley, 1977), using four hour old plasma, have suggested similar findings. Attempts to repeat the experiments on the comparison of "rapid" and "slow" thaw in Oxford produced inconsistent results due to variability in the Factor VIII levels, particularly for the "rapid" thaw pools, even though in these experiments the inter-donor Factor VIII variation had been eliminated by using aliquots from a 12-donor pool of plasma.

The inconsistency was attributed to the lack of expertise in the timing of the thawing process. While it is clear that in one RTC the "rapid" thaw process produces benefits, the process appears to be more critical than the "slow" thaw, and the benefits of this procedure have to be obtained with practice. Moreover, in many RTCs, "rapid" thaw would not be an advantage taking the work of the department into account and it will have to be shown to be of considerable benefit before its introduction is to be considered.

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The selection of plasma for the preparation of cryoprecipitates from donors with higher than average Factor VIII levels should lead to increased Factor VIII activity in those cryoprecipitates. In association with the double centrifugation experiments in Oxford (section 3.5.3), the Factor VIII levels of the starting plasma were determined. It was shown that the Factor VIII activity in the cryoprecipitates was in close correlation with that in the plasmas from which they were derived ($r = 0.95$). Unfortunately, in the exercise designed to investigate the possibility of selection along these lines, the result was particularly low assaying cryoprecipitates in the unselected group and cryoprecipitates of only average activity in the specially selected group. It is doubtful if selection of donors for the preparation of cryoprecipitates could ever be a practical proposition when one considers the number of cryoprecipitates prepared annually in England and Wales and the considerable increase in workload and logistic problems that prior screening of donors would entail.

As has been mentioned previously (section 3), several factors limit the value of a study such as the one undertaken. The most important are the inter-donor variability of Factor VIII activity and variables within the various RTCs, of which the most difficult to control is the volume of starting plasma. The latter is clearly important and illustrated by the fact that in the preliminary survey the Lancaster Centre had the lowest Factor VIII activity in the cryoprecipitates submitted for assay. At that time only 380 ml of blood was being collected at each donation. Subsequently the volume collected had been increased to 425 ml and the mean Factor VIII activity of six group O and six group A cryoprecipitates has increased from 58 iu per pack to 106 iu per pack.

The magnitude of the 'normal' variation in Factor VIII activity from donor to donor is such that, using pools of two group O and two group A cryoprecipitates, and comparing four such pools of one type with four similar pools of another type, the mean difference between the two effects being studied would have to be 35% or more to be discernible as significant at the 5% level. In order to investigate the factors influencing Factor VIII activity in cryoprecipitates, further experiments will have to be undertaken using aliquots of pools of plasma and it is recommended that for certain parameters these are carried out.

Nevertheless, the study has been valuable in that it has provided information that conditions of centrifugation, time of freezing and probably the age of the plasma up to 18 hours do not materially affect the Factor VIII activity of the cryoprecipitates.

The experiments also suggest that CPD is the anticoagulant of choice for cryoprecipitates prepared from plasma up to six hours old but probably has no advantage over ACD for cryoprecipitates prepared from overnight plasma (18 hours). Rapid thawing, if it can be controlled, also appears to be an advantage and further investigations are needed to assess this factor.

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TABLE I

NO. DONATIONS USED TO PREPARE CRYOPRECIPITATES PER ANNUM

REGION	PRESENT DAY	% Total DONATIONS	FUTURE ESTIMATE
Newcastle	15,000	12.5	13,000
Leeds	10,000	8.3	-
Sheffield	11,000	8.7	-
Cambridge	7,100	9.7	-
Edgware	50,000	31.3	-
Brentwood	10,800	8.0	-
Tooting	23,000	10.2	decreasing
Oxford	600	0.7	decreasing
Bristol	15,000	8.7	-
Birmingham	42,000	26.3	-
Manchester	16,000	15.2	-
Lancaster	2,500	88.5	-
Liverpool	20,000	18.2	-
Southampton	17,700	26.2	-
Cardiff	17,000	22.4	16,000
Total	257,700	15.0	

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TABLE II

DISTANCE OF SESSIONS FROM WHICH BLOOD IS USED FROM RTC FOR CRYOPRECIPITATE

	Within Centre	5 miles	5–25 miles	26–50 miles	50 miles	Special Van	Comments regarding geography or other special features of the Region
Newcastle	NO		SELECTION				Only 4% sessions within 30 miles of RTC
Leeds		few	almost 100%				Several large towns within 25 miles of RTC
Sheffield						100%	
Cambridge		40–50%		25%	25%		Predonimenly ural area
Edgware	few		almost 100%				High density population with traffic problems
Brentwood		←	100%	→			Considerable traffic problems
Tooting		←	100%	→			Dormitory area, poor road conditions
Oxford		12%	29%	60%	8%		Widely scattered urban areas
Bristol		60%	30%	10%			50% sessions exceed 50 miles from RTC
Birmingham		few	almost 100%				Distances small but heavy traffic
Manchester	50%	20%	30%				Densely population area near RTC
Lancaster		10%	20%	50%	25%		Scattered ural area with five towns
Liverpool		←	100%	→			75% sessions on detachment
Southampton		10%	73%	17%			several large towns 25–30 miles from RTC
Cardiff			70%	30%			Scattered rural area with a few towns

REGION	INITIAL CENTRIFUGATION PLASMA					CENTRIFUGATION TO HARVEST CRYOPRECIPITATES		
	CENT. TYPE	CENT. HEAD	Full Speed rpm g	Time at full speed mins.	Temperature of bowl °C	Full Speed rpm g	Time at full speed mins.	Temperature of bowl °C
Newcastle	MSE	4-pl angle	4000 3500	20	+ 4°C	4000 3500	15	+ 4°C
Leeds	MSE	6-pl s/o	2500 2075	15	+ 4°C	2500 2075	15	+ 4°C
Sheffield	MSE	6-pl	2500 2075	15	+ 4°	2500 2075	10	+ 4°
Cambridge	MSE	6-pl	2500 2075	30	+ 4°C	2500 2075	15	+ 4°C
Edgware	MSE Damon Sorval	6-pl s/o ——"—— 4-pl s/o	2500 2075 4200 4850 5000 6450	15 7 5	+ 4°C	2500 2075	15	+ 4°C
Brentwood	MSE	6-pl angle	4000 3850	4	+ 4°C	4000 3850	4	+ 4°C
Tooting	MSE	6-pl s/o	2500 2075	9	20°C	2500 2075	5	+ 4°C
Oxford	MSE	6-pl s/o 4-pl angle	2500 2075 4500 4500	30 10	+10°C	1900	30	+ 4°C
Bristol	MSE	6-pl s/o	4500 4800	8		4500 4800	9	+ 4°C
Birmingham	MSE	4-pl angle	5000 4800	5	+ 4°C	5000 4800	5	+ 4°
Manchester	MSE	6-pl s/o	2500 2075	20	+ 5°	2500 2075	20	+ 5°
Lancaster	MSE	6-pl s/o	2500 2075	20	+ 4°	2500 2075	15	+ 4°
Liverpool	MSE	6-pl angle	4000 3850	7	+4° - 6°	4000 3500	3	+4° - 6°
Southampton	MSE	6-pl s/o	2500 2075	25	+ 4°	None	-	ambient RT
Cardiff	MSE	4-pl angle 6-pl s/o	4500 5000 2500 2075	7½ 15	-14°	4500 2500 2075	7½ 15	+ 4°

KEY

pl = places in centrifuge head
s/o = swing out head
angle = angle head

TABLE IV

TIME TAKEN FROM DONATION TO FREEZING OF PLASMA EXPRESSED AS A PERCENTAGE OF TOTAL DONATIONS

REGION	4 Hours	4 - 6 Hours	18 Hours
Newcastle			100%
Leeds	25%	75%	
Sheffield	100%		
Cambridge	40%	60%	
Edgware	20%	80%	
Brentwood		50%	50%
Tooting	80%	20%	
Oxford		100%	
Bristol		100%	
Birmingham	25 - 30%	70 - 75%	
Manchester	100%		
Lancaster	20%	80%	
Liverpool	25%	75%	
Southampton	100%		
Cardiff	80%	20%	
Total	103,040 (40%)	129,460 (50%)	25,800 (10%)

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TABLE V

PREPARATION OF CRYOPRECIPITATE - FREEZING AND THAWING OF PLASMA

	PLASMA FREEZING		STORAGE PRIOR TO THAWING		THAWING OF PLASMA		
REGION	Temp °C	Time (mins)	Time (hrs)	Temp °C	Bath Temp °C	Cold Room Temp °C	Time Hours
Newcastle	-70	20	-	-	8 °C	-	2½
Leeds	-60 to -70	10	-	-	-	+4	12-14
Sheffield		7	-	-	-	+4	18
[illegible]ridge	-70	8	-	-	-	+4	12
Edgware	-50 to -60	30	-	-	-	+4	18
Brentwood	-70	20-25	-	-	-	+4	16-18
Tooting	-65	20	-	-	-	+4	up to 21
Oxford	-30	-	up to 3 weeks	-30	-	+4	20
Bristol		50	up to 1 week	-25	-	+4	16
Birmingham	-60 to -70	10-15	-	-	-	+4	16-18
Manchester		30	-	-	-	+4	16-18
[illegible]ster		15	-	-	-	+4	15-18
Liverpool		30	occasionaly 72 hours	-40	-	+4	13
Southampton	-70	15	-	-	-	+4	18
Cardiff		10-15 approx.	-	-	-	+4 to +6	18-24

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TABLE VI

1. Selection of donors

Bristol cryos : 'Routine' cf. 'Paediatric'

Block No.	iu Factor VIII per cryo	
	Routine	Paediatric
1	26.7	70.0
2	22.0*	72.2
3	49.7	94.3
4	46.5	85.4
Geometric Mean Potency	34.1	79.9
S.D. of log potencies expressed in antilog form as a percentage	49.8%	15.2%

*Only one group O and one group A cryo were used in the preparation of this pool.

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TABLE VII

2. Choice of Anticoagulant

Birmingham cryos : ACD cf. CPD

Block No.	iu Factor VIII per cryo	
	ACD	CPD
1	80.4	99.8
2	45.3	75.6
3	99.9	111.3
4	57.5	108.3
Geometric Mean Potency	67.6	97.7
S.D. of log potencies expressed in antilog form as a percentage	42.0%	19.3%

71/77

TABLE VIII

3. Age of plasma

Liverpool, Brentwood and Manchester cryos : "< 4 hour", "4-6 hour" and "18 hour" plasma as source material.

Block	iu Factor VIII per cryo		
	< 4 hour	4-6 hour	18 hour
LIVERPOOL	138.3 92.4 85.8 178.7	133.7 124.3 135.6 122.2	
Geometric Mean	118.3	128.8	
BRENTWOOD		104.5 73.5 54.1 75.9	94.8 72.5 103.9 99.7
Geometric Mean		74.9	91.9
MANCHESTER	98.1 80.5 70.8 69.5		97.2 56.8 77.2 72.5
Geometric Mean	79.0		74.6
Corrected Treatment Mean	91.4	88.7	96.9

71/74

TABLE IX

4. Anticoagulant plus age of plasma

Manchester cryos : ACD cf. CPD. "< 4 hour" cf. "18 hour" plasma.

Block No.		iu Factor VIII per cryo	
		ACD	CPD
1 2 3 4	< 4 hour plasma	66.6* 129.1 89.2* 63.0*	97.2 93.2 138.7 99.8
	Geometric Mean	83.4	105.6
	S.D. of log potencies expressed in antilog form as a percentage	38.9%	20.0%
1 2 3 4	18 hour plasma	87.5 61.7 101.9* 83.1	68.1 86.4 91.3 79.8
	Geometric Mean	82.2	80.9
	S.D. of log potencies expressed in antilog form as a percentage	23.4%	13.6%

*Only one group O and one group A cryo were used in the preparation of this pool.

(71/72)

TABLE X

5. Comparison of conditions for the centrifugation of whole blood to separate plasma for the preparation of cryoprecipitates.

(a) Edgware cryos : Comparison of different rotor speeds.

Block	iu Factor VIII per cryoprecipitate		
	Mistral, 2500 rpm	Damon, 4200 rpm	Sorval, 5000 rpm
1	97.7	77.6	86.2
2	99.4	115.4	91.8
3	93.3	85.2	98.6
4	70.2	80.7	93.6
Geometric Mean	89.3	88.6	92.4
S.D. of log potencies expressed in antilog form as a percentage	17.6%	19.8%	5.7%

71/79

TABLE XI

5. Comparison of conditions for the centrifugation of whole blood to separate plasma for the preparation of cryoprecipitates.

(b) Oxford cryos : Comparison of different centrifugation times at the same speed.

Block	iu Factor VIII per cryoprecipitate	
	9 mins at 2500 rpm	30 mins at 2500 rpm
1	88.2	87.8
2	83.2	82.1
3	59.7	86.8
4	103.1	95.1*
Geometric Mean	82.0	87.8
S.D. of log potencies expressed in antilog form as a percentage	25.8%	6.2%

*Only one group O and one group A cryo were used in the preparation of this pool.

71/80

TABLE XII

5. Comparison of conditions for the centrifugation of whole blood to separate plasma for the preparation of cryoprecipitate.

(c) Oxford cryos : Plasma separated by single centrifugation at 4000 rpm cf. plasma centrifuged twice at 4000 rpm.

Pool No.*	iu Factor VIII per cryoprecipitate	
	Single centrifugation	Double centrifugation
1	88.9	83.3
2	97.1	103.3
3	107.4	89.2
4	112.7	108.6
5	107.2	91.0
6	67.6	60.7
7	87.8	91.3
8	93.0	100.6
9	115.2	109.9
Geometric Mean Potency	96.3	91.8
S.D. of log potencies expressed in antilog form as a percentage	18.1%	19.9%

*Each pool comprised one group O and one group A cryo.

71/81

TABLE XIII

6. Comparison of conditions for freezing plasma donations prior to production of cryoprecipitate.

Sheffield cryos : 7 minutes at ≃ -80°C cf. 15 minutes at ≃ -80°C.

Block No.	iu Factor VIII per cryoprecipitate	
	7 mins @ -80°C	15 mins @ -80°C
1	45.1	87.2
2	73.7	67.4
3	86.5	57.9*
4	72.0	58.8
Geometric Mean Potency	67.4	66.9
S.D. of log potencies expressed in antilog form as a percentage	32.4%	20.8%

*Only one group O and one group A cryo were used in the preparation of this pool.

71/82

TABLE XIV

7. Comparison of conditions for thawing plasma donations to produce cryoprecipitates.

(a) Newcastle cryos : 18 hour plasma, rapid thaw,
cf. 18 hour plasma, slow thaw,
cf. 4 hour plasma, rapid thaw.

Block No.	iu Factor VIII per cryoprecipitate		
	18 hour plasma, rapid thaw	18 hour plasma, slow thaw	4 hour plasma, rapid thaw
1	92.9	66.0	107.1
2	79.4	66.4	125.8
3	95.0	73.1	100.7
4	92.1	56.7*	84.6
Geometric Mean Potency	89.7	65.3	103.5
S.D. of log potencies expressed in antilog form as a percentage	8.5%	11.1%	17.8%

*Only one group O and one group A cryo were used in the preparation of this pool

71/83

TABLE XV

7. Comparison of conditions for thawing plasma donations to produce cryoprecipitates.

(b) Oxford cryos : Rapid thaw cf. slow thaw of 12 plasma 'donations' prepared by pooling 12 donations of fresh plasma and dividing the pool into 12 parts.

Block No.	iu Factor VIII per cryoprecipitate	
	Rapid thaw	Slow thaw
1	107.8	101.9
2	54.1	97.1
3	123.2	93.4
4	125.0	99.0
5	124.0	101.5
6	114.2	83.3
Geometric Mean Potency	104.1 (118.6)	95.8 (95.6)
S.D. of log potencies expressed in antilog form as a percentage	38.4% (6.7%)	7.8% (8.8%)

The figures in brackets ( ) are the mean potency and S.D. obtained when the results in block 2 are omitted.

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TABLE XVI

7. Comparison of conditions for thawing plasma donations to produce cryoprecipitates.

(c) Oxford cryos : Rapid thaw cf. slow thaw of ACD cf. CPD plasma.

Block No.		iu Factor VIII per cryoprecipitate	
		Rapid thaw	Slow thaw
1 2 3 4 5 6	ACD Plasma	111.9 74.7 126.5 118.0 112.5 98.6	97.0 105.5 84.3 93.1 94.1 90.1
	Geometric Mean Potency	105.6	93.8
	S.D. of log potencies expressed in antilog form as a percentage	20.7%	7.8%
1 2 3 4 5 6	CPD Plasma	192.6 62.9 95.1 91.1 71.5 128.3	134.2 135.3 148.8 118.8 107.5 98.8
	Geometric Mean Potency	99.4	122.7
	S.D. of log potencies expressed in antilog form as a percentage	50.3%	16.8%

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REFERENCES

Burka, E.R., Puffer, T., and Martinez, J., (1975a)

The influence of donor characteristics and preparation methods on the potency of human cryoprecipitate.

Transfusion 15: 323-328

Burka, E.R., Harker, L.A., Kasper, C.K., Kevy, S.V., and Ness, P.M., (1975b)

A protocol for cryoprecipitate production.

Transfusion 15: 307-311

Graybeal, F.Q., Mooreside, D.F., and Langdell, R.D., (1969)

Clotting factor activity in cryoprecipitates and supernatant plasma prepared from blood collected into ACD, ACD-adenosine, CPD and CPD-adenosine and from plasma collected by plasmapheresis.

Transfusion 9: 135-144

Kasper, C.K., Myhre, B.A., McDonald, J.D., Nakasako, Y., and Fienstein, D.I., (1975)

Determinants of Factor VIII recovery in cryoprecipitate.

Transfusion 15: 312-322

Pool, J.G., (1967)

The effect of several variables on cryoprecipitated Factor VIII (AHG) concentrates.

Transfusion 7: 165-167

Slichter, S.J., Counts, R.B., Henderson, R., and Harker, L.A., (1976)

Preparation of cryoprecipitated Factor VIII concentrates.

Transfusion 16: 616-626

Stranberge, J.N., Gruhe, M.C., Tanaka, K., Chalos, M.K., and Aster, R.H., (1970)

A comparison of Factor VIII activity in cryoprecipitates prepared from ACD and CPD plasma.

23rd Ann. Meeting. AABB. p. 115

Vermeer, C., Soute, B.A.M., Ates, G., and Brummelhuis, H.G.J., (1976)

Contributions to the optimal use of human blood: VII. Increase of the yield of Factor VIII in four-donor cryoprecipitate by an improved processing of blood and plasma.

Vox Sang. 30: 1-22

Wensley, R.T., (1977)

Personal communication

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Addendum

5 litre packs of fresh frozen plasma for the preparation of Dried Factor VIII Concentrate by the Blood Products Laboratory

Although the preparation of 5 litre packs of "fresh-frozen" plasma was not within the formal terms of reference of the Cryoprecipitate Survey we thought it might be of interest to record relevant data concerning them while visiting the RTCs as part of the Cryoprecipitate Survey.

Summary of observations:

(1) Only two centres (for different reasons) give the preparation of 5 litre packs priority over local cryoprecipitate.

(2) In most centres 5 litre packs take second place to cryoprecipitate with respect to

(a) distance of sessions from centre.

(b) time from donation to freezing (most of the blood was stored overnight at +4°C prior to separation; in one centre 18 hour blood separated in the morning could be left to the afternoon before pooling).

(c) selection of donors - one centre rejected "fatty plasmas" for cryoprecipitate but included them in 5 litre packs.

(d) general interest taken by staff

(3) Two centres use predominantly CPD blood; all other ACD.

(4) Centrifugation conditions are very variable (as for cryoprecipitate).

(5) Grant freezers are used throughout, but nominal time of freezing is variable (70-100 minutes). In some centres actual times could be less, especially where staff thought the sole object of the freezer was to get the pack to hold the desired shape. Experiments by G.W.R. Dike in Oxford RTC confirmed that, in a pre-cooled Grant freezer, 75 minutes should suffice to lower the temperature at the centre of the pack to -25°

(6) Few centres had written instructions concerning the technical details.

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