

Therapeutic Materials used in the Treatment of Coagulation Defects

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Replacement therapy with coagulation factors has been extensively reviewed (Rizza, 1976; Urbaniak and Cash, 1977) but with little emphasis on distinctions between concentrates manufactured in different ways. Bidwell et al (1976) have described methods of manufacture in some detail. We will try to show what motivates fractionators to prepare concentrates by different methods and what effect this may have on the choice of product for a particular mode of treatment.

Some coagulation factor deficiencies can be treated successfully with plasma, preferably fresh-frozen plasma. Transfusions of whole blood should be given only when the circulating blood volume has been greatly diminished after a major bleed and the alternative use of red cell concentrates plus a specific factor concentrate should always be considered first. Table 1 lists the half-lives, haemostatic levels and in vivo recoveries of the coagulation factors whose deficiencies are commonly encountered. From these data it may be predicted that plasma levels of fibrinogen, factors X, XI, XII and XIII might be raised to haemostatic levels by the daily infusion of one litre FFP or less, a conclusion broadly confirmed by clinical experience. In addition, although only quite low plasma levels of factor V can be achieved by infusion of plasma, this is the only way factor V-deficient patients can be treated. Potent concentrates of some of these factors (e.g. X and XIII) are available as alternatives to whole plasma and the choice should be made according to the particular clinical circumstances. Especially where high levels of short-lived coagulation factors must be maintained, e.g. before and immediately after surgery, there is a risk of hypervolaemia resulting from repeated infusions of whole blood or plasma.

Plasma may be the logical choice if only a low level of the deficient factor is to be achieved, if more than one coagulation factor (or plasma protein) is deficient, or if some unusual risk attaches to the use of the alternative concentrate in that patient. Further, if the patient requires treatment only very infrequently, single-donor plasma may expose him to a lower risk of hepatitis than a more effective concentrate made from large pools of plasma.

Table 1. *In vivo* recoveries, half-lives and haemostatic levels of coagulation factors

| Factor | Concentration in FFP (u/ml) | Immediate recovery in circulation, % of theoretical ^a | Half-life (hours) ^{a b} | Plasma level for haemostasis (u/ml) ^{a c} | Plasma level (u/ml) after infusion of 1000 U (70 kg patient, completely deficient) | | |
|------------|-----------------------------|--|----------------------------------|--|--|-------|--------|
| | | | | | 0 h. | 12 h. | 24 h. |
| II | 0.9 | 50 | 72 | 0.40 | 0.17 | 0.16 | 0.13 |
| V | 0.3 | 50 | 15 | 0.15 | 0.17 | 0.09 | 0.05 |
| VII | 0.9 | 100 | 4 | 0.10 | 0.34 | 0.04 | < 0.01 |
| VIII | 0.8 | 70 | 8–12 | > 0.40 | 0.24 | 0.12 | 0.06 |
| IX | 0.9 | 40 | 12–24 | > 0.40 | 0.14 | 0.10 | 0.07 |
| X | 0.9 | 50 | 50 | 0.10 | 0.17 | 0.15 | 0.13 |
| XI | 0.9 | 90 | 60 | 0.30 | 0.31 | 0.28 | 0.23 |
| XII | 0.9 | | 60 | | | | |
| XIII | 0.9 | 50–100 | 100–300 | 0.02 | 0.17 | 0.16 | 0.16 |
| Fibrinogen | 3 mg/ml | 50 | 100 | 0.5 mg/ml | | | |

^a Firm data are lacking for some of the rarer deficiencies and in general the most pessimistic values have been quoted.

^b Half life is defined here as the time taken for the concentration of the factor in plasma to fall to half the concentration measured immediately after infusion of the dose.

^c Wide variations in 'haemostatic level' are reported for different patients with deficiency of the same factor and the level may differ according to the degree of injury, e.g. surgery, treatment of haemarthrosis or prophylaxis only. Moderately pessimistic levels have been selected, appropriate to surgery, but even these levels may have to be exceeded in individual cases.

High plasma levels of coagulation factors can be reached and maintained only with concentrates, the potency and safety of each batch is documented, no blood-group matching is required and there is little risk of hypervolaemia. Concentrates are made from large pools of plasma, commonly between 500 and 5000 donations. On the one hand, this increases the risk of transmitting viruses, e.g. hepatitis B virus undetected in one or more donations, but the increased risk is significant only for patients treated infrequently with blood products; other hypothetical risks, such as an increased chance of encountering some rare plasma antigen, are not confirmed in practice. On the other hand, any potential hazard of a single donation is diluted by pooling; in our experience the number of unexplained adverse reactions initiated by concentrates is less than that associated with single-donor cryoprecipitates or plasma, and concentrates may generally be given to patients of any blood group (but see the section below on hazards of factor VIII concentrates).

In preparing concentrates, purification of the desired factor is pursued only as far as is necessary to provide sufficient potency and solubility. Even when the selectivity of a fractionation stage is very high, a little of the desired factor is discarded with the unwanted fraction, and every added stage results in mechanical losses on wet equipment and the exposure of labile proteins to a denaturing environment. Many proteins are susceptible to activation or digestion by proteases present in plasma extracts during processing, or become less stable on dilution or removal of protective proteins such as albumin or protease inhibitors. Purification therefore is intended only to remove specific contaminants which may render the desired factor unstable or unsafe to administer, and to reduce the concentration of total protein or major contaminants which would limit the solubility and therefore the potency of the concentrate.

If plasma containing high concentrations of the desired factor is difficult and expensive to obtain, or if poor technology results in a poor process yield of the factor, or there is a high demand for the factor because of the relative numbers of potential recipients and donors, a rational policy for the best use of scarce resources will favour a high-yielding concentrate, even if it is less soluble or less easily infused than alternative concentrates. If the required factor is relatively stable, recoverable in high yield from plasma without affecting the recovery of other components, and if congenital deficiency of the factor is rare, it may be appropriate to sacrifice yield in the interest of obtaining a better product. Even in the former case, clinical need may dictate the use of a highly potent but low-yielding concentrate, e.g. to prepare a factor VIII-deficient patient for major surgery. This occasional need should not lead to indiscriminate use of the low-yielding concentrate for less demanding cases.

Freeze-dried concentrates are more stable than fresh plasma, frozen plasma or frozen cryoprecipitate. Each batch is assigned an expiry date beyond which it is not intended to be used. A concentrate is usually safe to use after its formal expiry date but it may have lost some potency. The potency stated on the label is valid on the day of assay, which will frequently predate issue of the batch by a month or more, but the concentrate can usually be expected to retain at least 90 per cent of the stated potency by the expiry

date, provided it is stored at the recommended temperature. Most concentrates have also been tested for stability after re-dissolving the dried product, and a warning to use within a certain period after solution is usually a precaution against bacterial contamination rather than instability.

As licensing authorities require ever more rigorous guarantees of safety and efficacy of 'biological' medicines, freeze-dried concentrates acquire further advantages over plasma or products made in small pools. Concentrates may be dispensed in 50 to 1000 vial lots from a single reservoir, and in all the processes associated with a batch great care is taken to ensure that each vial is in every significant way representative of the entire batch. The potency of each vial from a homogeneous batch can be assigned with confidence from replicate assays, usually on several vials, which encourages effective and economical use of concentrates. The results of tests on random samples from such a batch therefore carry much more conviction than those on the same number of vials, each of which has been produced by discrete processing of single donations or a small pool of donations. Important safety tests, for example those for bacterial pyrogen, cannot be justified for 'batches' consisting of one bottle and sampling for sterility tests in such 'batches' must be carried out retrospectively or at the risk of contaminating the bottle.

FACTOR VIII CONCENTRATES

Single-donor or small-pool cryoprecipitate

The peculiar solubility of factor VIII in plasma at low temperatures is very widely exploited for the production of factor VIII concentrates, either from single donations of plasma in plastic packs or bottles or as the first stage in the fractionation of very large pools of plasma. Cryoprecipitate is separated from other plasma proteins by freezing the plasma and thawing it to the minimum temperature consistent with the melting of all ice in a reasonable time. Perfect mixing of solid and liquid phases is never achieved and the supply of heat required to carry ice through its melting point must be carefully controlled lest it raise the temperature of the suspension, or local regions of the suspension, above the temperature at which cryoprecipitate will dissolve. It is difficult to determine experimentally whether there is a critical temperature below which factor VIII is insoluble in plasma, but it seems likely that it has a finite solubility at all temperatures above freezing point and that in practice the amount redissolved in plasma is a function of both time and temperature.

Many factors may affect the yield and specific activity of factor VIII recovered by cryoprecipitation but such systematic studies as have been carried out do not agree on the relative importance of these factors. The amount of factor VIII delivered to a patient is dependent on the amount of factor VIII in the original donation of FFP, the efficiency of its recovery by cryoprecipitation, its stability during frozen storage and the efficiency of its re-solution, pooling with other cryoprecipitates and injection. It is therefore not surprising that the concentration and total amount of factor VIII in each cryoprecipitate should be highly variable. Under carefully controlled experi-

mental conditions, the yield of factor VIII from plasma may be approximately 70 per cent, but the yield obtained in Transfusion Centres making a hundred or more cryoprecipitates each day is usually much less than this. In a recent survey among Regional Transfusion Centres in the United Kingdom, the yield of factor VIII varied from 40 to 127 i.u. per 180 ml plasma 'donation', with a mean of about 70 i.u. Variation of factor VIII yields within each centre, only partly attributable to variation in plasma factor VIII content between group O and group A donors, explains why clinicians tend to use more packs of cryoprecipitate for each patient than would be justified by calculations based on the average yield; prudence demands the assumption of a factor VIII content nearer the minimum of the range than the mean and many patients effectively receive more cryoprecipitate than would be required if the factor VIII content of the dose were more predictable.

Variation in the factor VIII content of a dose of cryoprecipitate diminishes as the number of cryoprecipitates included in a pool increases, and a higher standard of quality control becomes more economically feasible. Some national fractionation laboratories adopt the policy of pooling 2 to 12 redissolved cryoprecipitates and freeze-drying them to yield a relatively stable concentrate with a potency of about 5 i.u./ml. Some laboratories determine the factor VIII content of each bottle before freeze-drying, while others declare a mean 'expected' factor VIII content based on analysis of randomly selected bottles. The price of this increased certainty in factor VIII content is the exposure of the patient to a greater number of plasma donations than could be attained with single cryoprecipitates. On the other hand, quality control on such pools cannot be as extensive as that which can be expended on samples of a product dispensed from a single large pool.

The preparation of small-pool cryoprecipitate continues because, despite its variability and inconvenience, it provides the maximum gross yield of factor VIII without the capital cost associated with a specialized fractionation centre, and because plasma processed to cryoprecipitate in a Transfusion Centre is immediately available for treatment there or in the local Haemophilia Centre.

Freeze-dried concentrates

Figure 1 illustrates some of the options available to the fractionator preparing factor VIII concentrates but does not purport to describe the fractionation scheme for any product. Some concentrates are known not to fit neatly into any of these groups, and some companies do not divulge their methods of manufacture. The figure illustrates the general proposition that, as the number of purification stages is increased to provide a more soluble and potent concentrate, a diminishing yield of factor VIII may be expected.

Group 1 encompasses single-donation cryoprecipitate, freeze-dried cryoprecipitate and Cohn fraction I. Fraction I is precipitated from plasma at about eight per cent ethanol, pH 6.8 to 7.2 and -2°C . A higher proportion of factor VIII is precipitated from plasma by this method than by cryoprecipitation alone, but a large burden of other proteins, including almost all the fibrinogen in plasma, is also precipitated. The precipitate is therefore poorly soluble

at 2 to 4 i.u./ml and factor VIII in this solution is rather unstable. Since the solution is extremely difficult to filter, it is usually made by expensive and time-consuming aseptic technique. Fraction I is not readily purified to group 2 and 3 concentrates, but it serves as an intermediate in the Blombäck (1958) process which exploits the relative solubilities of factor VIII and unwanted contaminants in buffers containing glycine, citrate and ethanol; the 'washed' fraction I-0 meets the specification of group 2 concentrates.

Group 2 concentrates are derived from cryoprecipitate or 'ethanol-assisted' cryoprecipitate. In some authors' experience, the addition of only three per

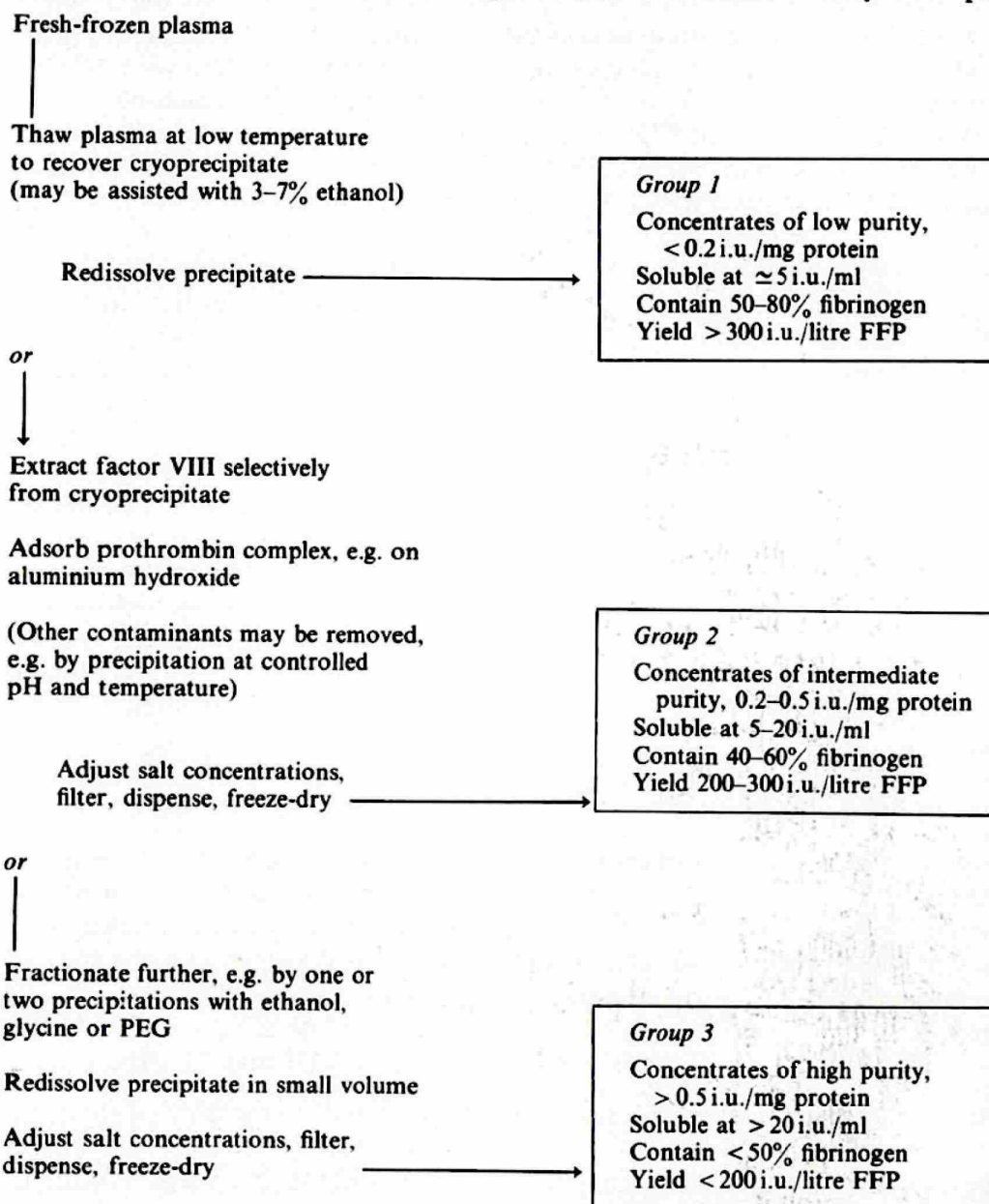


Figure 1. Alternative schemes for production of factor VIII concentrates of 'low', 'intermediate' and 'high' purity (specific activity).

cent ethanol during the thawing of plasma gives a more reliable yield of factor VIII at the expense of increased precipitation of other proteins, especially fibrinogen; others have found no significant increase in yield of factor VIII or too little to balance greater difficulties in filtering the product or re-dissolving it after freeze-drying. Both kinds of precipitates are easier to purify further than is Cohn fraction I. Most manufacturers use some of the following purification stages:

1. *Washing.* If the crude cryoprecipitate is washed in cold distilled water or saline, some of the supernatant plasma trapped in the precipitate may be removed without appreciable loss of factor VIII. One manufacturer has exploited the principle of cold washing much further to obtain two-fold purification and is able to extract factor VIII from the washed cryoprecipitate at an unusually high concentration (Wickerhauser, Mercer and Eck-enrode, 1978).
2. *Extraction of factor VIII from cryoprecipitate.* Factor VIII may be dissolved selectively from cryoprecipitate by suspending it in buffers of low ionic strength. The pH, ionic strength, concentration of specific ions, temperature, protein concentration and rate and duration of agitation are all important variables in determining the maximum extraction of factor VIII and the minimum extraction of unwanted contaminants. The final potency of most group 2 concentrates is largely determined at this stage by the volume of extraction buffer, which is in turn influenced by the solubility of the cryoprecipitate; e.g. cryoprecipitate formed in the absence of ethanol and subjected to a cold wash may be soluble in less buffer than an unwashed cryo-ethanol precipitate. On the other hand, if too little solvent is used, the high viscosity of the solution may exaggerate losses at a later stage in processing. If the extraction is successful, more than 10 per cent of the total protein in cryoprecipitate (including much aggregated fibrinogen which, if not removed, can complicate sterile filtration and other stages) may be removed at the expense of about five per cent of the factor VIII.
3. *Adsorption of prothrombin complex.* If factors II, VII, IX and X and any activated factors which may have formed in earlier processing are completely removed from the extract containing factor VIII and fibrinogen, the final concentrate will not clot spontaneously. Such stability is a major advantage during processing and after re-solution of the dried product. Aluminium hydroxide, prepared as a sterile and pyrogen-free gel suspension, is less toxic than the barium sulphate often used in the laboratory, but citrate should not be added to the extraction buffer since appreciable concentrations of aluminium ions may be dissolved in citrate solutions. Again, pH, temperature and duration of adsorption as well as the adsorptive properties of the gel influence the adsorption of the coagulation factors. If adsorption is inefficient the concentrate may be less stable, but if too much gel is used there may be a heavy loss of factor VIII trapped in the sedimented gel.
4. *Cold precipitation.* Some manufacturers include at this stage, or just before gel adsorption, a simple precipitation of contaminants from the extract at controlled pH and temperature. Conditions may be varied to yield a

highly soluble product at the expense of much factor VIII, or one which is less rapidly soluble but recovered in high yield. However, since contaminants and not the factor VIII are precipitated at this stage it has little influence on the potency at which the product is dispensed.

5. *Finishing operations.* The last stages of producing factor VIII concentrate are intended to provide a sterile, stable, freeze-dried powder which can be re-dissolved rapidly for injection as a physiologically safe solution. One way to enhance the potency of a concentrate is to dispense the final solution at an ionic strength approximately half that of plasma and re-dissolve the dried product in distilled water to half the dispensed volume, if the solubility of the product permits. With such manoeuvres in mind, the fractionator adjusts the pH and the concentrations of sodium chloride, sodium citrate and any other stabilizers at this stage.

Since factor VIII does not withstand high temperature, and since many adsorptive filters inactivate it, solutions containing factor VIII can be satisfactorily sterilized only by filtration through membranes, e.g. of cellulose esters, which can remove all bacteria and their spores; some viruses are not removed. The filtrate is collected in a sterile receiver, from which it is dispensed into sterile vials, frozen and freeze-dried with aseptic precautions. Since some factor VIII is adsorbed by membrane filters and losses on wet equipment increase with increased potency of the extract, there are advantages in removing contaminants which may impede filtration on a small area of membranes. Careful freezing and freeze-drying should result in negligible loss of factor VIII activity.

Group 3 concentrates are derived from one of the intermediate stages in the processing of group 2 concentrates. They may incorporate two precipitations, the first to remove contaminants less soluble than factor VIII and the second to precipitate factor VIII and leave more soluble contaminants in the supernatant. The second precipitation is important because it allows factor VIII to be concentrated by re-solution in a small volume of any convenient solvent, but the first precipitation may greatly improve the solubility of the second precipitate.

Ethanol, glycine and polyethylene glycol of molecular weight 4000 to 6000 daltons have been used alone and in combination for selective precipitation of factor VIII. One of the few methods published in detail is the 'high purity' method of Newman et al (1971).

The starting material is an adsorbed extract of a cryo-ethanol precipitate. Polyethylene glycol is added to a final concentration of about three per cent at pH about 6.3 and the suspension centrifuged. The precipitate contains very little factor VIII but a high proportion of fibrinogen and other contaminants. As in the cold precipitation of group 2 concentrates, the combination of PEG concentration and pH at the first precipitation may be varied to emphasize either purity or yield of factor VIII in the final product. Factor VIII is then precipitated from the supernatant by increasing the concentration of PEG and adjusting pH; this second stage does not result in great purification but the precipitate can be re-dissolved in about one-fifth the volume of the intermediate extract, or one-fiftieth of the original plasma

volume. However, most manufacturers recommend re-solution to a potency of about 30 i.u./ml or less, presumably to discourage too rapid infusion and too much loss on the wet vial and infusion equipment.

The only major advantage of group 3 concentrates over those of group 2 is their high potency, which may not always be crucially important. No manufacturer of factor VIII claims that the risk of transfusing isoagglutinins or the virus of hepatitis B is greatly reduced by further precipitations, and there is no firm evidence that the recovery or survival of factor VIII *in vivo* is higher after transfusions of more potent concentrates. However, the proportion of fibrinogen to factor VIII may be usefully reduced. On the debit side, group 3 concentrates may contain residues of the precipitants used in the final stages and the ratio of factor VIII coagulant activity to factor VIII antigen may be less than that of group 2 concentrates, suggesting a greater burden of denatured, inactive factor VIII. It must be emphasized that no adverse reactions have been reliably traced to either of these features of re-precipitated concentrates. The main obstacle to a universal switch from groups 1 and 2 to group 3 concentrates is that the yield of factor VIII from the latter is substantially smaller.

In communities where human plasma is seen as an element in the economy of whole blood, the supply of fresh frozen plasma suitable for production of factor VIII and other protein concentrates is linked with the demand for other blood components such as red cell concentrates. Unpaid donors are seldom lacking, but it may be considered too expensive or unethical to bleed donors if all components may not be fully utilized. By the same argument, of course, whole blood should form a much smaller proportion of each Blood Transfusion Service's inventory than it does today. Commercial pharmaceutical companies purchase plasma, collected mainly in plasmapheresis centres, with few of the concerns of a comprehensive Blood Transfusion Service, as simply an expensive ingredient in a manufacturing process. The fee paid to plasmapheresis donors is the major component of the high cost of commercial concentrates. Had these concentrates not been available during the last few years, haemophiliacs in many countries—even those with an apparently flourishing National Transfusion Service—would have received grossly inadequate treatment, and the quality and convenience of commercial concentrates has set a high standard for national fractionation centres. However, their availability has also diverted attention from the likely reward of investing equivalent sums in national Blood Transfusion and Fractionation Services. The Haemophilia Centre Directors of the United Kingdom calculated in 1977 that their patients could be treated adequately with approximately 50 million units of factor VIII annually, or about 15 000 to 20 000 i.u. per registered haemophiliac. The number of donations of fresh frozen plasma required to produce that factor VIII as intermediate-purity concentrate is quite within the reach of a modern Blood Transfusion Service (Hässig and Lundsgaard-Hanson, 1978). However, if more than the small proportion of factor VIII supplies necessary to treat the most demanding cases were converted to group 3 concentrates, it might be necessary to resort once more to dependence on imported products.

Treatment of patients with inhibitors of factor VIII

In some patients congenitally deficient in fibrinogen, factor IX and most frequently factor VIII, circulating antibodies to the deficient factor have appeared after replacement therapy. Infusions of factor VIII are inactivated by the antibody over a period of minutes to hours and the factor may be detectable in the circulation only briefly after infusion or not at all.

Since the aim is to induce haemostasis before further antibody has been produced in response to infusions, the most potent concentrate should be used. Even if large doses produce no detectable level of factor VIII in plasma, bleeding may be stopped or reduced, however briefly. This approach has been recommended for the treatment of acute bleeding and even for prophylaxis (Brackmann and Gormsen, 1977). If large doses do not result in clinical improvement a high level of antibody continues to be produced; recourse must then be made to simple supportive measures such as immobilization of the affected limb. There is also a heterogeneous group of regimens which have been reported as beneficial to some patients but which carry such risks that they are not usually used until conventional measures have been exhausted.

1. If the patient has not developed antibodies to animal factor VIII after former exposure, and if his antibody to human factor VIII does not cross-react with animal factor VIII, bovine or porcine factor VIII concentrates may be of value. Factor VIII is the only animal coagulation factor produced for clinical use.
2. Topical application of thrombin, fibrin foam or snake venoms may arrest bleeding locally, e.g. at the site of a wound.
3. Steroids or immunosuppressive drugs may be given alone or in combination with infusions of the deficient factor (Shapiro, see Chapter 10).
4. Some patients with antibodies to factor VIII, not responding to other treatments, have benefited from infusions of factor IX concentrates (Kurczynski and Penner, 1974), which were said to contain 'factor VIII inhibitor bypassing activity'. Some patients appear to have been helped even by concentrates normally issued with confidence for haemophilia B, which should contain extremely low concentrations of activated factors, but most success has been reported from the use of concentrates which have been specially selected for their content of activated factors ('FEIBA') or even specially activated ('Autoproplex'). It must be emphasized that activated factor IX concentrates contain potent coagulants which would render them unsafe for use in any other circumstances. The constituents responsible for clotting may be factors VIIa, IIa, IXa, Xa, perhaps in combination with their proenzymes, but neither this nor any other hypothesis has been tested using synthetic mixtures of the separated factors. Standards do not exist for some of the potential coagulants and there is variation in clinical effect from batch to batch. At least one clinical trial is now in progress to define more clearly the safety and effectiveness of this empirical form of therapy.

Factor VIII concentrates in von Willebrand's disease

Patients with von Willebrand's disease lack factor VIII antigen and factor VIII coagulant activity but can regain some of the latter if factor VIII antigen is replaced by infusion of a concentrate. All group 1 and 2 concentrates of factor VIII appear to supply the missing factor but it is reported that at least some group 3 concentrates of high potency may not correct the bleeding time in von Willebrand's disease (Blatt et al, 1976; Green and Potter, 1976). Since the patient's coagulant response to infusions of factor VIII is usually high and sustained, group 1 and 2 concentrates should be chosen unless there are overriding considerations, e.g. of blood volume.

Some hazards associated with the use of factor VIII concentrates

Although differing in methods of manufacture, factor VIII concentrates share certain characteristics. The ratio of factor VIII coagulant activity to factor VIII antigen is always less than that for plasma, suggesting that factor VIII in concentrates is either selected from the range of molecules present in plasma, or is partially denatured. The question has arisen whether denatured factor VIII might be likely to elicit the production of circulating antibodies to factor VIII, but most authors have concluded that a relatively constant percentage of haemophiliacs in any population is susceptible, and acquires inhibitors after treatment with any therapeutic material, including plasma.

Concentrates made from unselected donors contain a relatively constant ratio of factor VIII to anti-A and other isoagglutinins and may cause haemolysis when used in the intensive treatment of a haemophiliac of blood group A or AB (Orringer et al, 1976). Although tentative limits have been set for isoagglutinins in factor VIII concentrates, no standard method for quantitation or standard anti-A reference material has been agreed and it is meaningless to compare titres of anti-A obtained in different laboratories. Even in one laboratory, it is not usual to determine the relative concentrations of IgG and IgM anti-A, which may not be equally important in causing haemolysis.

Factor VIII is present as only a small proportion of the proteins in even the most highly purified concentrates, but 'allergic' reactions should be seen less frequently in response to concentrates than to plasma or cryoprecipitate. All concentrates, except that of manufacturer F (see Appendix), contain large amounts of fibrinogen, usually accounting for 30 to 60 per cent of the total protein. Fibrinogen is not the only protein limiting solubility of factor VIII concentrates but the most potent concentrates are likely to have a low ratio of fibrinogen to factor VIII. Very high doses, even of group 3 concentrates, may elevate plasma levels of fibrinogen and its split products, but levels up to 5 mg fibrinogen per ml plasma have no harmful effects.

CONCENTRATES CONTAINING FACTORS IX, II, X AND VII

Congenital deficiency of factor IX (haemophilia B, Christmas disease) has an incidence about 12 per cent that of haemophilia A and cannot be distinguished from the latter on clinical grounds. Although the half-life of factor IX is longer than that of factor VIII, the immediate recovery of factor IX after infusion is usually less than half the theoretical value, possibly because of rapid diffusion into extravascular spaces. This poor recovery makes it difficult to maintain haemostatic levels of factor IX in patients by infusing whole blood, plasma or cryoprecipitate supernatant and their use is restricted to particular cases where the patient might be unusually vulnerable to one of the risks associated with freeze-dried concentrates.

Factors II, IX and X, and to a lesser extent factor VII, have similar charge properties and tend to be recovered together when whole plasma is fractionated, e.g. by precipitation with salts or organic solvents, or by adsorption and elution. For that reason methods of preparing all four factors will be considered together. All four factors are fairly stable in plasma and are not removed by cryoprecipitation or precipitation of Cohn fraction I. Whole plasma, cryoprecipitate supernatant and supernatant from Cohn fraction I have all been used as starting materials for factor IX production. During large-scale cold ethanol fractionation of plasma for the production of IgG and albumin, all four factors are concentrated into fractions III and IV of Cohn methods 6 and 9 or into corresponding fractions of alternative fractionation schemes. These fractions are attractive as a source of factor IX since they do not involve any modification of long-established schemes for other valuable products and since they are usually discarded, but most fractionators have abandoned their use since they are partly denatured, contain activated forms of the coagulation factors and, if the original plasma contained hepatitis B surface antigen, this presumed marker for the infective virus is found in high concentrations in these fractions.

Most concentrates containing factor IX are now made from cryoprecipitate supernatant using an ion-exchanger to adsorb from the plasma the desired factors, which are then selectively eluted with buffers of increasing ionic strength (Figure 2). The choice of ion-exchanger is based primarily on the relative concentrations of factors II, VII, IX and X required in the product. The ion-exchangers in common use are DEAE-cellulose and DEAE-Sephadex. Factor VII is less well adsorbed from diluted plasma at near-neutral pH than are factors II, IX and X, but binding capacity increases in the order of the charge density of the ion-exchangers. The choice of ion-exchanger has many consequences which must be evaluated as a whole.

DEAE-Sephadex is attractive because it can adsorb a high proportion of all four factors from cryoprecipitate supernatant or the supernatant from Cohn fraction I without dilution, i.e. it can adsorb at quite high ionic strength. However, this means that even higher ionic strength must be used to elute factors II, IX and X and the eluate must be desalted by dialysis, ultrafiltration or precipitation. Also, if HB_sAg is present in plasma it may be adsorbed under approximately the same conditions as is factor VII, and some attempts to reduce the potential infectivity of the product by extensive

washing of the ion-exchanger lead to significant losses of factor VII. DEAE-Sephadex is mechanically weak in the swollen state and changes dimensions and flow rate in response to changes in ionic strength and pH. Consequently, it can only be used on the production scale in a batch mode, and the superior resolution of column chromatography cannot be exploited with this exchanger. The difficult recycling of such 'soft' ion-exchangers for repeated use also has cost implications, and indeed some manufacturers use fresh adsorbent for every batch.

Fresh-frozen plasma
 ↓
 Cryoprecipitation on large scale—precipitate used
 to recover factor VIII
 (Optional removal of Cohn F.I with 8% ethanol)
 ↓
 Adjust pH and ionic strength of supernatant
 ↓
 Adsorb with ion-exchanger (or inorganic adsorbent)

Alternative methods for elution of ion-exchanger

| <i>Purpose of stage</i> | <i>Column elution (e.g. DEAE-cellulose)</i> | <i>Batch elution (e.g. DEAE-Sephadex)</i> |
|--|--|--|
| Elution of weakly adsorbed contaminants | Suspend 'loaded' ion-exchanger in buffer of low salt concentration, pack into chromatographic column | Suspend 'loaded' ion-exchanger in buffer of low salt concentration, and wash several times on a wide filter mesh |
| Elution of some or all of factor VII, and possible reduction of HB _s Ag concentration | Elute column with buffer of higher salt concentration | Wash adsorbent several times on filter with buffer of higher salt concentration |
| Elution of factors II, IX, X | Elute column with second increment of salt concentration | Wash adsorbent several times with second increment of salt concentration |
| Preparation for issue | (<i>Eluate already in approximately isotonic solution at 20–40 i.u. factor IX/ml</i>) Sterilize eluate, dispense and freeze-dry | Remove salts from eluate by dialysis and concentrate by freeze-drying <i>or</i> Desalt and concentrate by ultrafiltration or reprecipitation Sterilize, dispense and freeze-dry |

Figure 2. Alternative schemes for production of factor IX concentrates using anion-exchangers.

DEAE-cellulose was the first true ion-exchanger to be used for the recovery of factor IX and it has been retained by several manufacturers because of its relatively low binding capacity and its good mechanical properties. After dilution of the cryosupernatant with about 0.3 volumes of water, factors II, IX and X may be almost completely adsorbed by DEAE-cellulose; factor VII is only partially adsorbed. Dilution of the plasma makes subsequent recovery of IgG and albumin more expensive, but does not interfere with its efficiency. HB_sAg is less strongly bound to DEAE-cellulose than to DEAE-Sephadex. Weakly bound factor VII may be recovered in relatively poor yield by washing the 'loaded' ion-exchanger with a buffer of low ionic strength. Factors II, IX and X can be eluted from DEAE-cellulose at near-physiological ionic strength and the eluted factors can be injected without desalting, particularly if chromatographic methods are used. DEAE-cellulose does not change dimensions very much with changes in pH and ionic strength, and when the 'loaded' ion-exchanger has been removed from the plasma it may be suspended in a buffer of low ionic strength and packed into a chromatographic column. The column is then developed frontally with buffers of increasing ionic strength; factor VII and then factors II, IX and X together may be eluted in very sharp peaks, each occupying about 40 per cent of the volume of the column or about one per cent of the volume of the adsorbed plasma. Reconcentration of the eluate can therefore be avoided, as well as desalting.

DEAE-Sephadex has a binding capacity and mechanical properties intermediate between those of DEAE-cellulose and DEAE-Sephadex. It is particularly useful for the preparation of factor VII separately from factors II, IX and X, since it adsorbs factor VII more strongly than does DEAE-cellulose but can be packed into a chromatographic column like the latter. Frontal elution with a buffer of relatively low ionic strength yields a factor VII concentrate of 20 to 30 u/ml potency without further desalting and concentration (Dike, Griffiths and Tadman, 1977).

Semi-specific inorganic adsorbents may be used in a batch mode similar to that described for DEAE-Sephadex. Barium sulphate and aluminium hydroxide are little used now for the preparation of clinical concentrates because of the known or potential toxicity of residues from the adsorbent remaining in the product. The first successful therapeutic concentrate of factor IX, introduced in 1959, was the original Paris 'PPSB' in which tricalcium phosphate was used as adsorbent (Soulier et al, 1969). Since citrate is a specific eluant for this adsorbent, plasma has to be taken from blood collected into EDTA anticoagulant, which does not fit well into current strategies for the use of blood components. Since all four factors are well adsorbed and the eluate from the adsorbent is further purified by ethanol fractionation, the product contains all the four factors at a high potency, usually over 20 u/ml. This product has an excellent record of safety and although it is expensive and inconvenient to produce, its use might be preferred if a patient had persistent adverse reactions to a range of concentrates made by ion-exchange.

The source material, choice of adsorbent and the mode of elution are not the only variables influencing the composition of the eluate. The buffers used

to wash or elute the adsorbent sequentially usually contain two or more of the following ions: citrate to chelate calcium ions (which might initiate activation), phosphate to provide good buffering and contribute to the control of calcium ions, and chloride to make up the required ionic strength. The particular choice of buffer solutions will affect the concentration and ratio of coagulation factors eluted, the pH and ionic content of the eluate (and therefore the need to de-salt it before sterilizing and dispensing), the concentration of contaminating proteins and the extent of activation which occurs during processing.

Further processing may be undertaken with the aim of de-salting, concentrating or purifying the eluate. De-salting can be effected by dialysis, gel filtration, ultrafiltration (which may also concentrate all proteins) or precipitation, which may be selective enough to increase the specific activity of the coagulation factors as well as their concentration. A de-salted eluate may be concentrated by bulk freeze-drying and even de-salting may be omitted if a volatile eluant buffer is chosen ('Konyne'). There may be other motives for re-precipitation of the eluate than a simple desire to achieve a higher potency. An interesting example is the two-stage re-precipitation of eluate from an ion-exchanger with PEG 4000 under controlled conditions of pH, specific ion concentrations, protein concentration and PEG concentration ('Supernine'); this selectively removes from factor IX most of the HB_{Ag} which may be present in the eluate (Johnson et al, 1976) and also some substances which, in the presence of calcium ions, can initiate rapid production of factor Xa and thrombin in the concentrate. Another manufacturer uses PEG to refractionate an eluate obtained after adsorbing an extract of Cohn fraction IV₁ on calcium phosphate, but it is not known whether HB_{Ag} or activated factors are removed ('Proplex').

All these secondary processes result in losses of yield and, although the plentiful supply of starting material makes this relatively unimportant in the economy of plasma, the cost of the concentrate is affected. Extensions of processing time may also be costly, and the increased number and complexity of manipulations offer more opportunities for denaturation of coagulation factors on surfaces and their apparently spontaneous activation to factors IXa, Xa and thrombin.

In 1974, the ICTH reviewed a number of reports of thromboembolic complications and one death following the infusion of certain batches of factor IX concentrates made in the U.S. ('Proplex' and 'Konyne'). Those at special risk appeared to be the newborn, patients with liver disease and congenitally deficient patients undergoing major surgery (Kasper, 1975). The danger of thromboembolism in haemophilia B patients without obvious complicating factors had perhaps the most disturbing implications for therapy, and experience with these relatively new concentrates in the U.S. contrasted with the long successful record of factor IX concentrates in Europe (e.g. Bidwell et al, 1976). A number of semi-specific tests for the presence of activated factors in concentrates had been developed (Kingdon et al, 1975) and it was believed that the offending concentrates contained more of these activated factors than did others with no history of provoking

thromboembolic disorders. When concentrates from many different manufacturers were screened by these tests, all were found to have some content of activated factors but there were consistent and very wide variations in their concentrations.

The suspect concentrates were thrombogenic in rabbits, in the 'stasis' thrombus model of Wessler, Reiner and Sheps (1959), but the clotting tendency of some unheparinized concentrates could be diminished by the addition of heparin to the dose. There is no evidence that heparin protects human patients from the adverse effects of factor IX concentrates, particularly in liver disease, but on the basis of the animal model the Committee recommended the addition of heparin to all concentrates at about 5 to 10 units per ml (Ménaché and Roberts, 1975). It is not obvious that heparin can stabilize freeze-dried factor IX concentrates, especially those which contain little or no functional antithrombin, and insufficient heparin is added to have a significant systemic anticoagulant effect in the patient. Heparin may inhibit clotting locally in the injected vein, but that has not been the pattern of thromboembolic complications. On balance, the addition of heparin to factor IX concentrates can do little harm, but the user should not expect it to render completely safe a concentrate activated during processing. One argument against the addition of heparin is that it reduces the sensitivity and discrimination of existing *in vitro* tests for activated factors and might allow an unsatisfactory batch to pass a simplified routine programme of safety tests. While tests for activated factors are quite sensitive, they are not very specific or quantitative. It is still not known which activated factor or combination of factors is most likely to induce thromboembolism in patients or even laboratory animals. Some of the current hypotheses have been reviewed by White et al (1977), who suggest the addition of both heparin and antithrombin III to factor IX concentrates.

Concentrates made ostensibly in very similar ways using the same starting material, ion-exchanger, chromatographic method and buffer ions, may vary very widely in their distributions of activated factors. Apparently minor differences in procedure may alter the balance of activating and protecting mechanisms during processing. Even working within one well-defined method of fractionation, we have found significant differences in the distribution of activated factors and other protein contaminants which depend on the pH of adsorption, the duration of adsorption, the method used to separate the loaded adsorbent from cryoprecipitate supernatant, the temperature and constituent ions of the eluting buffers and small (five per cent) changes in the selection of the factor IX-containing fraction.

In the absence of quantitative standards for the specific measurement of activated factors and their relationship to clinical risk, the safest concentrates must be regarded as those which, besides performing well in routine *in vitro* tests, have consistent properties from batch to batch and have a long record of safety when used in a wide variety of clinical circumstances and in high doses. It is therefore extremely important that all cases of thromboembolism following the use of factor IX concentrates continue to be reported to the manufacturer and in the medical and scientific literature.

THERAPEUTIC MATERIALS IN CONGENITAL DEFICIENCIES OF OTHER COAGULATION FACTORS

Factor II

Like factor X, factor II is present in most concentrates of factor IX at a potency of 20 to 40 units/ml. Although factor II has a long half-life it is incompletely recovered after infusion and a high plasma level (approximately 0.4 units per ml) is required for haemostasis. This level is difficult to attain by infusions of plasma and a concentrate should be preferred in most cases.

Factor X

Most concentrates of factor IX also contain factor X at a potency of about 20 units/ml, but unless high plasma levels must be reached, e.g. prior to surgery, or frequent treatment is required, plasma may be preferred because of its lower risk of transmitting hepatitis B. Although initial recovery of infused factor X is incomplete, its long half-life might encourage prophylaxis with weekly or fortnightly doses of concentrate.

Factor VII

Factor VII is present in many concentrates of factor IX, usually at a lower concentration than factors IX, II and X. Other concentrates of factor IX are prepared by methods which exclude factor VII but the latter may be recovered, after the adsorption of factors IX, II and X, by a second adsorption, e.g. with DEAE-Sephadex or aluminium hydroxide gel. Factor VII has the shortest half-life of all the coagulation factors but the 'haemostatic level' is usually quite low in congenital deficiency and some patients do not bleed even after surgery. If bleeding occurs, concentrates restore haemostasis more reliably than plasma.

Fibrinogen

Congenital deficiency of fibrinogen is seldom complete and rarely causes spontaneous bleeding—a continuing reproach to most theories of normal coagulation.

Most of the fibrinogen prepared for clinical use is intended for the therapy of acquired deficiencies, e.g. in postpartum bleeding, and a small amount is labelled with radioisotopes of iodine as an aid to diagnosis, e.g. of deep vein thrombosis.

Fresh frozen plasma contains about 3 g fibrinogen per litre. The survival of infused fibrinogen is unusually dependent on the rate of consumption in coagulation and therefore on the severity of injury to the congenitally deficient patient. Normal haemostasis is usually achieved at plasma levels exceeding 0.5 g/l.

Single-donor cryoprecipitate contains approximately 30 per cent of the fibrinogen in whole plasma and carries the same low risk of transmitting hepatitis as does plasma. Cryoprecipitate should therefore be the first choice

for any congenitally deficient patient who does not experience frequent reactions to plasma or cryoprecipitate. We have been unable to trace any report of a patient requiring frequent treatment and prophylaxis, but in this context one would contemplate the use of Cohn fraction I, a purified fibrinogen derived from the latter or any of the fibrinogen-rich factor VIII concentrates which may be readily available, if somewhat expensive.

Factor V

Human factor V is unstable in plasma or freeze-dried plasma and laboratory procedures for its purification give a very poor yield. There is no freeze-dried concentrate for therapeutic use. Until recently, factor VIII was believed to be inherently very unstable and factor V may also prove to be unstable only in particular environments. Fresh frozen plasma contains approximately 0.6 units of factor V per ml, the initial recovery after infusion is quite high and haemostasis is achieved at 10 to 15 per cent of normal levels, even after surgery. However, the short half-life of factor V dictates frequent infusions.

Factor XI

The high initial recovery of infused factor XI and its long half-life usually make it possible to maintain haemostatic levels with fresh frozen plasma, even after surgery. No specific concentrate of factor XI is manufactured, but some batches of one type of factor IX concentrate ('Proplex') contain useful concentrations of factor XI; this has been used effectively in a haemorrhagic crisis (Bick, Adams and Radack, 1974).

Factor XII, Fleaujac, Fitzgerald and Fletcher factors

Congenital deficiencies of these factors, which operate in the contact phase of coagulation, are not usually associated with spontaneous bleeding. Rare bleeding, e.g. after surgery, is treated with fresh frozen plasma. No concentrates are available.

Factor XIII

About 100 cases of congenital deficiency of factor XIII have been reported, and the incidence may be between 1 in 10^6 and 1 in 10^7 . Intracranial haemorrhage seems to be commoner than in haemophilia A or B. Fortunately, factor XIII is relatively stable in plasma and has a very long post-transfusion half-life. Haemostasis may be achieved by infusion of whole plasma, and cryoprecipitate, containing about 60 per cent of the factor XIII in plasma at about 3 units/ml potency, is obviously useful. Cohn fraction I is also enriched in factor XIII but is usually made from pooled plasma and, apart from its stability in storage as a freeze-dried powder, has little obvious advantage over cryoprecipitate.

The persistence of infused factor XIII in the circulation makes prophylaxis attractive and continuing therapy justifies the use of a concentrate from

pooled plasma. In fact, the only concentrate of factor XIII available for therapeutic use is prepared from human placental blood, not venous plasma. This concentrate is stable in a freeze-dried state and is used at a potency of about 60 units/ml and a dose of about 10 to 15 units/kg (Losowski and Miloszewski, 1976). Such doses given prophylactically every one or two months have led to a dramatic improvement in the lives of congenitally deficient patients who appear to be liberated both from spontaneous bleeding and from dependence on a nearby Haemophilia Centre.

The potential hazards associated with this concentrate are the risk of transmitting hepatitis B, the possible antigenicity of placental tissue proteins or blood group antigens, and the possible toxicity or carcinogenicity of the acridine derivative, Rivanol, used as a precipitant in the manufacturing process. No such sequelae have been reported in five years' use of the concentrate in the treatment of congenital or acquired factor XIII deficiency.

It is unusual for any manufacturer to prepare and stock a concentrate which may be required for so few patients. The cost of developing a new concentrate and carrying out clinical trials in a small population is rising steeply and a flexible approach to the licensing of such concentrates for clinical trial may be in the best interests of patients with rare deficiencies.

TRANSMISSION OF HEPATITIS B

The risk of transmitting hepatitis with concentrates depends on the number of donations in the plasma pool and the care taken to exclude infective donations from the pool. Some fractionation methods distribute HB_sAg, a marker for hepatitis B virus, unevenly between fractions (Berg et al, 1972) and most concentrates of coagulation factors fortunately contain lower concentrations of HB_sAg than the plasma pools from which they are derived, but even concentrates with no HB_sAg detectable by the most sensitive methods may transmit hepatitis B. Since the best current tests detect about 10⁸ particles of virus per ml and a dose containing less than 10⁶ particles/ml may be infective, tests on final products are of little value. The dilution of a single infective donation in many hundreds or thousands of others reduces the efficiency of screening only the pool, and it is considered that screening of each donation by the most sensitive 'third generation' tests is essential to reduce the risk of transmitting hepatitis B.

With the possible exceptions of factor II and factor XIII, coagulation factors will not withstand the heat exposure required to inactivate hepatitis B in plasma or concentrates. Manufacturer G prepares a factor IX concentrate from plasma treated with β -propiolactone to inactivate virus. Specific precipitation of HB_sAg with PEG appears to be successful in laboratory tests, but success in reducing post-transfusion hepatitis also awaits clinical trials.

Few haemophiliac populations are regularly tested for recent exposure to infection, e.g. by serial measurement of antibody to core antigen of hepatitis B. The long incubation period between infection and the appearance of symptoms means that a suspect batch of concentrate has usually been completely

used before it can be firmly implicated in a cluster of cases and its recall ordered.

Disquieting reports of the incidence of chronic hepatitis in patients treated for haemophilia (Mannucci et al, 1975; Levine et al, 1977) are a reminder that the underlying problem has still to be solved and should be a spur to further improvements in the screening of plasma and the removal of virus from blood products. These warnings are particularly relevant to haemophilia B since the concentrations of activated factors in some factor IX concentrates currently available render them unsafe for use in patients with liver disease.

Despite such dangers, haemophiliacs and their medical advisers emphatically prefer adequate therapy with concentrates to the prospect of pain and crippling without treatment.

APPENDIX: SUMMARY OF COAGULATION FACTOR CONCENTRATES PRODUCED BY MAJOR MANUFACTURERS IN EUROPE, AMERICA AND AUSTRALIA

This list was compiled from information available in early 1978 and may be incomplete. Many of the listed manufacturers may not export their products.

United Kingdom

A. Blood Products Laboratory

(a) *Blood Products Laboratory, Elstree, Herts.*

Factor VIII, intermediate purity, incorporating cold precipitation. *Fibrinogen*. *Fibrin foam*. *Thrombin*.

(b) *Plasma Fractionation Laboratory, Churchill Hospital, Oxford.*

Factor VIII, intermediate purity, incorporating cold precipitation. *Factor IX* (IX, II and X only) by elution from DEAE-cellulose; heparinized. *Factor VII* by elution from DEAE-Sepharose; no heparin.

B. Scottish National Blood Transfusion Service, Protein Fractionation Centre, Edinburgh.

Factor VIII, intermediate purity. *Factor IX*, (i) 'DEFIX' (IX, II and X only), by elution from DEAE-cellulose; no heparin. (ii) 'Supernine', further purified by precipitation with PEG, on clinical evaluation only. (iii) 'PPSB', by elution from calcium phosphate and ethanol precipitation; heparinized. *Fibrinogen*.

Republic of Ireland

C. Blood Transfusion Service Board, Dublin.

Factor IX, (i) (IX, II and X only), by elution from DEAE-cellulose and ultrafiltration. (ii) (IX, II, X and VII), by adsorption of diluted plasma on DEAE-cellulose, elution and ultrafiltration.

West Germany

D. DRK Blutspendedienst Baden-Württemberg, Baden-Baden.

Factor VIII, high purity (PEG). *Factor IX*, (IX, II, X and VII), by elution from DEAE-cellulose and ethanol precipitation; heparinized.

E. DRK Blutspendedienst, Hagen.

Factor VIII, (i) small-pool freeze-dried cryoprecipitate. (ii) high purity (PEG). *Factor IX*, (IX, II, X and VII), by elution from DEAE-Sephadex and diafiltration; heparinized. *Fibrinogen*, (i) Cohn FI. (ii) high purity (PEG).

F. Behringwerke AG, Marburg/Lahn.

Factor VIII, (i) freeze-dried cryoprecipitate, may be supplied 'isoagglutinin-free' from selected donations. (ii) high purity, less than five per cent fibrinogen. *Prothrombin-concentrate*, (IX, II, X and VII); heparinized. *Fibrinogen*. *Bovine thrombin*.

G. Biotest-Serum-Institut, Frankfurt am Main.

Factor IX, 'PPSB-concentrate' (IX, II, X and VII) from plasma sterilized by β -propiolactone and eluted from DEAE-Sephadex; no heparin.

The Netherlands*H. Central Laboratory of the Netherlands Red Cross, Amsterdam.*

Factor VIII, small-pool freeze-dried cryoprecipitate. *Factor IX*, (IX, II, X and VII) by elution from DEAE-Sephadex and dialysis; no heparin. *Fibrinogen*.

France*J. Centre National de Transfusion Sanguine, Orsay-Courtabœuf, Paris.*

Factor VIII, (i) large-pool freeze-dried cryoprecipitate. (ii) high purity (PEG). *Factor IX*, 'PPSB', (IX, II, X and VII), by elution from DEAE-Sephadex and dialysis; heparinized. Original 'PPSB' using calcium phosphate no longer produced. Other concentrates containing these factors are being developed. *Fibrinogen*.

Switzerland*K. Central Laboratory of the Swiss Red Cross, Berne.*

Factor VIII, (i) small-pool freeze-dried cryoprecipitate. (ii) high purity (PEG). *Factor IX*, (IX, II, X and VII), by elution from DEAE-Sephadex; heparinized. *Fibrinogen*.

Austria*L. Immuno, Vienna.*

Factor VIII, 'Kryobulin', high purity; also available 'free of iso-agglutinins' or 'blood group compatible' by selection of plasma. *Factor IX*, (i) 'Bebulin' or 'Prothromplex' (IX, II and X only); < 1 u heparin/ml. (ii) 'Prothromplex Total' or 'Fulthromplex' (IX, II, X and VII); < 1 u heparin/ml. (iii) 'FEIBA' fraction for treatment of haemophilia A patients with inhibitors. *Factor VII* concentrate, by adsorption of DEAE-Sephadex supernatant on aluminium hydroxide gel. *Fibrinogen*.

Norway*M. Blood Bank, Ullevål Hospital, Oslo.*

Factor VIII, freeze-dried cryoprecipitate. *Factor IX*, (IX, II and X only), by elution from DEAE-cellulose.

Sweden

N. Kabi AB, S-11287 Stockholm.

Factor VIII, FI-O. Factor IX, 'Preconativ', (IX, II, X and VII), by elution from DEAE-Sephadex; no heparin. Fibrinogen. Antithrombin III, on clinical evaluation only.

Finland

P. Finnish Red Cross, Helsinki.

Factor VIII, (i) small-pool freeze-dried cryoprecipitate. (ii) intermediate purity concentrate. Factor IX, (IX, II, X and VII), by elution from DEAE-Sephadex and de-salting by gel filtration; no heparin. Fibrinogen.

Canada

Q. Connaught Laboratories Limited, Willowdale, Ontario.

Factor IX, (IX, II, X and VII).

United States of America

R. Abbott Laboratories, South Pasadena, California.

Factor VIII, 'Profilate', high purity.

S. Armour Pharmaceutical Company, Kankakee, Illinois.

Factor VIII, 'Factorate', intermediate purity.

T. Cutter Laboratories, Berkeley, California.

Factor VIII, 'Koate', high purity, re-precipitated with ethanol. Factor IX, 'Konyne', (IX, II, X and VII), by elution from DEAE-Sephadex and concentration by freeze-drying from volatile buffers; no heparin.

U. Hyland Division, Travenol Laboratories, Costa Mesa, California.

Factor VIII, 'Hemofil', 'Method 4', high purity, precipitated with PEG and glycine. Factor IX, (i) 'Proplex', (IX, II, X and VII), from Cohn FIV₁, elution from calcium phosphate and precipitation with PEG; heparinized. (ii) 'Autoproplex', activated concentrate for use in treatment of haemophilia A patients with inhibitors.

V. Michigan State Laboratories, Lansing, Michigan.

Factor VIII, intermediate purity, incorporating two-stage extraction of cryoprecipitate.

W. New York Blood Centre, New York.

Factor VIII, (i) 'Lyoc VIII', intermediate purity. (ii) 'Lyoc VIII/P', high purity (PEG).

Argentina

X. Instituto de Investigaciones Hematologicas, Buenos Aires.

Factor VIII, 'Fi-O-Ta', purified using tannic acid. Factor IX, (IX, II, X only), by elution from DEAE-cellulose. Fibrinogen, high purity (tannic acid).

Australia

Y. Commonwealth Serum Laboratories, Parkville, Victoria.

Factor VIII, high purity (PEG). Factor IX, (i) 'Prothrombinex' (IX, II, X only), by elution from DEAE-cellulose; heparinized. (ii) 'PPSB', by elution from calcium phosphate and ethanol precipitation; heparinized. Fibrinogen.

REFERENCES

- Berg, R., Björling, H., Berntsen, K. & Espmark, Å. (1972) Recovery of Australia Antigen from human plasma products separated by a modified Cohn fractionation. *Vox Sanguinis*, **22**, 1-13.
- Bick, R. L., Adams, T. & Radack, K. (1974) Surgical haemostasis with a factor XI-containing concentrate. *Journal of the American Medical Association*, **229**, 163-165.
- Bidwell, E., Dike, G. W. R. & Snape, T. J. (1976) Therapeutic Materials. In *Human Blood Coagulation, Haemostasis and Thrombosis* (Ed.) Biggs, R. pp. 249-309. Oxford: Blackwell Scientific.
- Bidwell, E., Rizza, C. R., Dike, G. W. R. & Snape, T. J. (1976) Clinical use of factor IX concentrates. *Thrombosis and Haemostasis*, **35**, 488-491.
- Blatt, P. M., Brinkhous, K. M., Culp, H. R., Krauss, J. S. & Roberts, H. R. (1976) Antihemophilic factor concentrate therapy in von Willebrand disease. Dissociation of bleeding time factor and ristocetin cofactor activities. *Journal of the American Medical Association*, **236**, 2770-2772.
- Blombäck, M. (1958) Purification of antihaemophilic globulin. I. Some studies in the stability of the antihaemophilic globulin activity in fraction I-0 and a method for its partial separation from fibrinogen. *Arkiv för Kemi*, **12**, 387-396.
- Brackmann, H. H. & Gormsen, J. (1977) Massive factor VIII infusion in haemophiliac with factor VIII inhibitor, high responder. *Lancet*, **i**, 933.
- Dike, G. W. R., Griffiths, D. & Tadman, A. J. (1977) Factor VII concentrate. *Thrombosis and Haemostasis*, **37**, 572-573.
- Green, D. & Potter, E. V. (1976) Failure of AHF concentrate to control bleeding in von Willebrand's disease. *American Journal of Medicine*, **60**, 357-360.
- Hässig, A. & Lundsgaard-Hansen, P. (1978) The procurement of blood and plasma for the production of components and derivatives within the frame of an integrated national blood programme. *Vox Sanguinis*, **34**, 257-260.
- Johnson, A. J., Semar, M., Newman, J., Harris, R. B., Brandt, D., Middleton, S. & Smith, J. (1976) Removal of hepatitis B surface antigen (HBsAg) from plasma fractions. *Journal of Laboratory and Clinical Medicine*, **88**, 91-101.
- Kasper, C. K. (1975) Clinical use of factor IX concentrates: report on thromboembolic complications. *Thrombosis et Diathesis Haemorrhagica*, **33**, 640-644.
- Kingdon, H. S., Lundblad, R. L., Veltkamp, J. J. & Aronson, D. L. (1975) Potentially thrombogenic materials in factor IX concentrates. *Thrombosis et Diathesis Haemorrhagica*, **33**, 617-631.
- Kurczynski, E. M. & Penner, J. A. (1974) Activated prothrombin concentrate for patients with factor VIII inhibitors. *New England Journal of Medicine*, **291**, 164-167.
- Levine, P. H., McVerry, B. A., Attock, B. & Dormandy, K. M. (1977) Health of the intensively treated haemophiliac, with special reference to abnormal liver chemistries and splenomegaly. *Blood*, **50**, 1-9.
- Losowsky, M. S. & Miloszewski, K. J. A. (1976) Management of patients with congenital deficiency of fibrin stabilising factor (factor XIII). Presented at the 16th International Congress of Haematology, Kyoto, 1966. *Excerpta Medica International Congress Series*.
- Mannucci, P. M., Capitanio, A., del Ninno, E., Colombo, M., Pareti, F. & Ruggeri, Z. M. (1975) Asymptomatic liver disease in haemophiliacs. *Journal of Clinical Pathology*, **28**, 620-624.
- Ménaché, D. & Roberts, H. R. (1975) Summary report and recommendations of the Task Force members and consultants. *Thrombosis et Diathesis Haemorrhagica*, **33**, 645-647.
- Newman, J., Johnson, A. J., Karparkin, M. & Puszkun, S. (1971) Methods for the production of clinically effective intermediate- and high-purity factor VIII concentrate. *British Journal of Haematology*, **21**, 1-20.
- Orringer, E. P., Koury, M. J., Blatt, P. M. & Roberts, H. R. (1976) Hemolysis caused by factor VIII concentrates. *Archives of Internal Medicine*, **136**, 1018-1020.
- Rizza, C. R. (1976) Coagulation factor therapy. *Clinics in Haematology*, **5**, 113-133.
- Soulier, J. P., Ménaché, D., Steinbuch, M., Blatrix, C. & Josso, F. (1969) Preparation and clinical use of PPSB (Factors II, VII, X and IX concentrate). *Thrombosis et Diathesis Haemorrhagica*, **35**, 61-72.

- Urbaniak, S. J. & Cash, J. D. (1977) Blood replacement therapy. *British Medical Bulletin*, **33**, 273-282.
- Wessler, S., Reiner, S. M. & Sheps, M. C. (1959) Biologic assay of a thrombosis-inducing activity in human serum. *Journal of Applied Physiology*, **14**, 943-946.
- White, G. C., Roberts, H. R., Kingdon, H. S. & Lundblad, R. L. (1977) Prothrombin complex concentrates: potentially thrombogenic materials and clues to the mechanism of thrombosis in vivo. *Blood*, **49**, 159-170.
- Wickerhauser, M., Mercer, J. E. & Eckenrode, J. W. (1978) Development of large scale fractionation methods. VI. An improved method for preparation of antihemophilic factor. *Vox Sanguinis*, **35**, 18-31.