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CRYOPRECIPITATE THERAPY IN HAEMOPHILIA

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Summary. *This paper describes experience over an 18-month period of a new preparation of factor VIII, cryoprecipitate, prepared by the Regional Blood Transfusion Service from human plasma. This relatively simple and inexpensive method selectively extracts the factor VIII leaving behind the other blood products for use in other ways.*

Replacement therapy in the treatment of haemophilia has been generally regarded as inadequate due to the difficulty in obtaining potent antihaemophilic globulin in sufficient amounts, and cryoprecipitate represents a useful advance.

Bleeding episodes in 25 patients with factor VIII deficiency have been treated with cryoprecipitate on 41 occasions and an assessment of the haemostatic effect of this material has been made in treating haemarthrosis, muscle haematomata and the management of major and minor surgical operations. Routine follow-up over a 6 month period was undertaken to determine the incidence of side effects. Two cases of hepatitis were found, possibly resulting from cryoprecipitate therapy. However, both patients had also received blood or fresh frozen plasma and the source of the hepatitis virus must remain in doubt. No factor VIII inhibitors were found despite repeated courses of therapy in several patients.

The advantages and disadvantages of cryoprecipitate are set out and its encouraging place in the management of haemophilic bleeding assessed.

THE control of bleeding in the haemophilic patient can only be effectively achieved by replacement of the missing plasma fraction, factor VIII (antihaemophilic globulin). The main sources of factor VIII are shown in Table I. All these therapeutic materials however have serious limitations and disadvantages and it is for this reason that many haemophiliacs receive replacement therapy which may be short of ideal. Though the issue is very difficult to assess objectively, Dormandy and associates (1967) considered that possibly only one fifth of haemophilic patients received treatment acceptable by modern standards.

The therapeutic problem is to raise the factor VIII content of the recipient's plasma to a haemostatic level and to maintain it there until the site of bleeding has healed. The haemostatic level of factor VIII may vary according to the problem. A level of 30 units per 100 ml. may be sufficient to secure haemostasis in a minor haemostatic challenge such as haematuria but 50 units per 100 ml. may well be required for a more serious injury such as extraction of several teeth.

To obtain a haemostatic level in a severe haemophiliac the factor VIII content of 3 to 5 litres of fresh plasma has to be ad-

Table I. Therapeutic materials in haemophilia.

Material	Expected rise in plasma factor VIII (with average dosage schedules)	Uses	Disadvantage
Whole blood Fresh plasma	Up to 5 units/100 ml.	Replacement of cells	Low potency
Fresh frozen plasma	Up to 20 units/100 ml.	Haemarthrosis Extraction of single tooth Haematuria	Increase in plasma vol. Low potency Expensive
Human factor VIII Lyophilised fraction of plasma	Up to 40 units/100 ml.	Multiple dental extractions Dangerous haematomata	Short supply Variation in potency Very expensive
Animal factor VIII (porcine or bovine)	Up to 200 units/100 ml.	Major trauma or surgery	Antigenic Thrombocytopenia Expensive

1 unit = amount of factor VIII present in 1 ml. of pooled fresh normal plasma.

ministered intravenously once every 24 hours; whole blood therefore is only of use to raise the haemoglobin value when blood has been lost. Plasma can be separated from the red cells of donor blood and maintained frozen at —20°C. until used; this provides fresh frozen plasma. Fresh plasma or fresh frozen plasma can be used but the large volumes of these unconcentrated agents which may be needed can pose major problems. Until recently, freeze dried fractions of human plasma provided the only human factor VIII concentrate. These are expensive to prepare requiring sophisticated fractionation equipment; in the process other valuable plasma fractions are lost.

Animal factor VIII van be prepared from bovine and porcine plasma. These materials are potent but are antigenic and if used for more than ten to fourteen days unacceptable reactions occur.

A major advance in the practical management of haemophilia has followed the observations of Pool and Robinson (1959) that fibrinogen and factor VIII precipitate out of plasma at low temperatures and can therefore be concentrated. Pool *et al.* (1964) and Hershgold *et al.* (1966) have shown that it is possible to prepare this cryoprecipitate for therapeutic purposes using simple apparatus. In view of the disadvantages of the previous sources of factor VIII, the application of the cryoprecipitate method of preparation to the practical management of haemophilia has been studied. In this paper the results are reported of our first 18 months experience of cryoprecipitate produced by the West of Scotland Regional Blood Transfusion Centre. During this period 2,100 packs of cryoprecipitate, prepared from the same number of blood donations have been infused.

MATERIALS AND METHODS

Preparation of cryoprecipitate

Cryoprecipitate was prepared according to the method of Pool and Shannon (1965). Processing was carried out on fresh blood and the separated plasma always frozen within three hours of collection. Blood from unselected donors is taken into Fenwal J. D. —2 packs (Baxter Laboratories Ltd.); this consists of a primary blood pack containing 75 ml. of acid-citrate dextrose and a transfer pack. The red cells are sedimented by centrifugation at 600 g. for 30 minutes at 4°C. and the supernatant plasma (about 280 ml.) expressed into the transfer pack. The red cells are then stored at 4°C. for use as 'packed cells'. The plasma is immersed in solid carbon dioxide-alcohol mixture at —79°C. for 20 minutes then placed in a 4°C. refrigerator and allowed to thaw for 18 hours. The plasma pack is then recentrifuged at 4°C. 600 g. for 35 minutes and the supernatant plasma in the transfer pack removed for albumin and immunoglobulin production except for 10 to 15 ml. which is left to reconstitute the cryoprecipitate. The packs are then stored at —70°C. The technique is illustrated in Figure 1.

Reconstitution

For use the cryoprecipitate is thawed in a 37°C. waterbath and the contents of the

Fig. 1. Cryoprecipitate prep
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transfer plastic pack; 3. Secon
dioxide, alcohol mixture; and
supernatant plasma has been

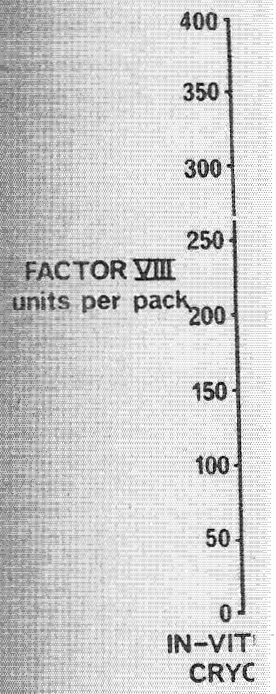


Fig. 2. *In vitro* Factor VIII a
packs showing the mean
standard deviation (79 unit

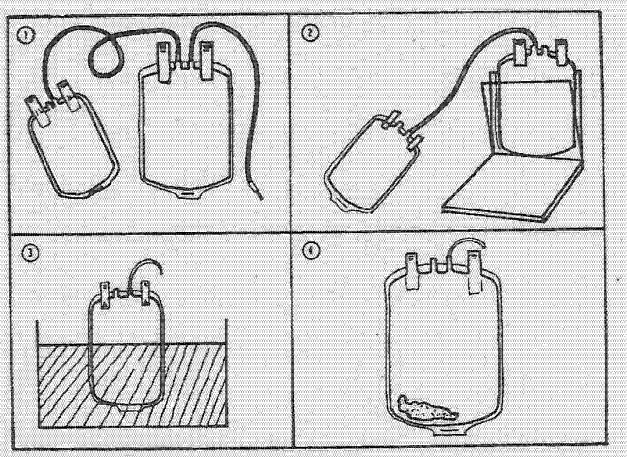


Fig. 1. Cryoprecipitate preparation. 1. Plastic primary and transfer pack; 2. Supernatant plasma being expressed from primary pack into secondary transfer plastic pack; 3. Secondary plastic pack being snap frozen in solid carbon dioxide, alcohol mixture; and 4. Button of cryoprecipitate which remains after supernatant plasma has been removed.

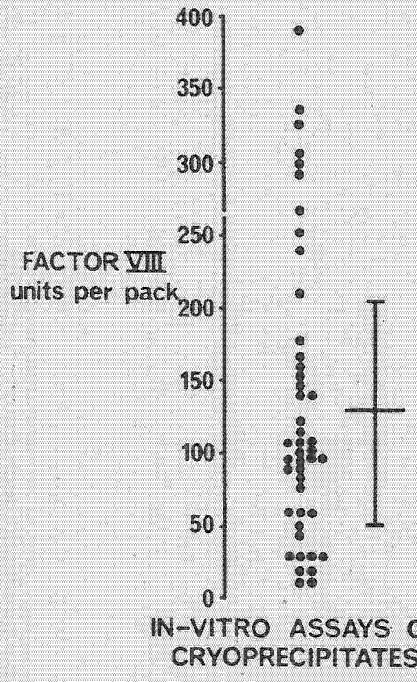


Fig. 2. *In vitro* Factor VIII assays of 45 cryoprecipitate packs showing the mean (130 units per pack) ± 1 standard deviation (79 units).

appropriate number of packs pooled using the pooling set designed by Davidson and Muir (1968). The average volume of cryoprecipitate was 21 ml. (mean of 786 packs).

Administration

As with other factor VIII concentrates, cryoprecipitate must be given intravenously. Intramuscular injection produces no significant rise in circulating factor VIII and large local haematomata form at the injection site (Pool *et al.*, 1966). In children and babies the required dosage is easily given by syringe (Bennett *et al.*, 1967) but in adults a conventional intravenous infusion is normally necessary because of the larger volumes.

Dosage

Dosage requirement varies depending on, *e.g.* the severity of the patient's defect, the type of bleeding and the type of operative intervention contemplated. For minor bleeding episodes a plasma factor VIII level of 30 units per 100 ml. has to be maintained and for major surgery a plasma level of 40 to 50 units per 100 ml. is necessary. This may be achieved by daily or twice daily infusion till healing is complete. (One unit of factor VIII activity is the amount present in one millilitre of fresh pooled normal human plasma (Pool & Shannon, 1965.)

Prentice *et al.* (1967) and Bennett *et al.* (1967) have used elaborate dosage schemes. However, due to the variability of factor VIII levels in individual packs (Fig. 2) and also the great variation in recovery of infused factor VIII in individual patients, we have used a simple empirical daily dosage scheme: for minor bleeding episodes 10 packs, for major episodes 20 packs, were given as a single rapid infusion. Factor VIII assays were performed after each infusion and if necessary further infusions were given.

Factor VIII assay

Plasma was obtained for factor VIII assay by the addition of 9 volumes of blood to 1 volume 3.8 per cent sodium citrate. Samples were centrifuged at 800 g for 10 minutes.

Samples of blood for factor VIII assay were taken before infusion and 10 minutes after infusion was completed. Assays were carried out as soon as possible and this was usually within 30 minutes of collection. Samples of cryoprecipitate concentrates were diluted in citrate saline 1 in 10 or 1 in 20 before assay to reduce the factor VIII level to approximately that of whole plasma.

Factor VIII was assayed by the method of Douglas (1962) which is a modification of the kaolin-partial thromboplastin time of Margolis (1958) and is similar to that described by Hardisty and MacPherson (1962). This assay is essentially a comparison of the corrective effect of normal plasma and the plasma under test on the partial thromboplastin time of haemophilic substrate plasma.

Using a pool of 9 fresh normal plasmas arbitrary dilutions are made which correspond to a range from 100 per cent to 5 per cent factor VIII. The test plasma is diluted in buffer in the same proportion as the 100 per cent standard.

Substrate haemophilic plasma (0.2 ml. aliquots) is pipetted into glass test tubes and 0.1 ml. amounts of the above dilutions added with 0.1 ml. of kaolin suspension (40 mg. kaolin/ml.) and incubated at 37°C. for 10 minutes to allow maximal contact activation. Equal volumes of platelet suspension and M/20 calcium chloride are added and the clotting times recorded. The clotting times of the standard are plotted against the dilutions of the standard using a log scale. Using this standard curve the clotting time of the test plasma can be converted to per cent factor VIII assuming that the pool represents 100 per cent factor VIII *i.e.*, 100 units per 100 ml.

Fibrinogen assay. This was carried out by the method of Ratnoff and Menzie (1951).

Plasminogen assay. This was carried out by the method of Remmert and Cohen (1949) as modified by Alkjaersig (1960).

Plasminogen activator. This was assessed by the euglobulin lysis time as described by McNicol and Douglas (1964).

Erythrocyte sedimentation rate. This was carried out as described by Dacie and Lewis (1963).

Red cell antibodies. These were sought by an indirect antiglobulin technique using a panel of cells containing the commoner red cell antigens. Blood samples were taken 6 weeks to 6 months after cryoprecipitate therapy.

Calculation of recovery *in vivo*. To assess the recovery of infused factor VIII in a patient we have used the criteria of Biggs and Macfarlane (1966). The method is described by Bennett *et al.* (1967), and the details are as follows:

$$\text{Dose in units} = \frac{\text{Vol. infused (ml.)} \times \text{factor VIII (\%)}}{100}$$

$$\text{Dose in units per kg. body weight} = \frac{\text{Dose in units}}{\text{Weight in kg.}}$$

$$\text{Expected rise in factor VIII (\%)} = \text{Dose in units per kg.} \times \text{constant (2.4)}$$

$$\text{Actual rise of factor VIII (\%)} = \text{Post infusion} - \text{pre-infusion level}$$

$$\text{\% Recovery} = \frac{\text{Actual rise in factor VIII \%} \times 100}{\text{Expected rise in factor VIII \%}}$$

CASE MATERIAL

Forty-one separate bleeding episodes in 25 patients were treated; 24 had haemophilia and one had von Willebrand's disease with a low factor VIII level. The patients treated have necessarily been selected and they represent the more serious bleeding incidents.

The various bleeding episodes treated are shown in Table II.

Table II. Patients treated with cryoglobulin precipitate. 41 bleeding episodes in 25 patients—24 with haemophilia; 1 with von Willebrand's disease

		Number of bleeding episodes
Haematomata/haemarthrosis		19
Gastrointestinal bleeding		6
Haematuria		5
Epistaxis		1
Surgery	1. Minor surgery	3
	2. Dental extraction	3
	3. Major surgery	4

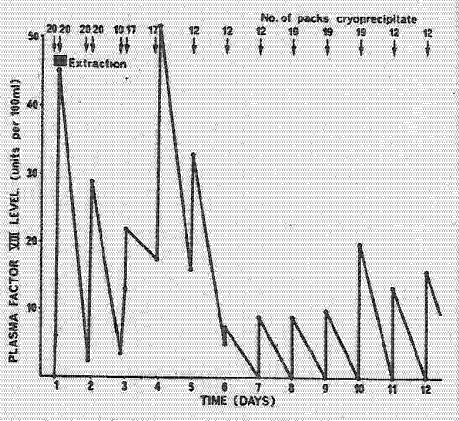
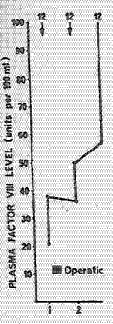


Fig. 3. Case 1. Plasma Factor VIII levels produced by cryoprecipitate infusions over a 12-day period following dental extractions. Each arrow indicates a separate infusion and the number of packs infused.



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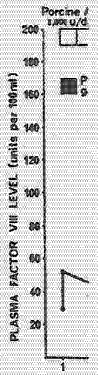

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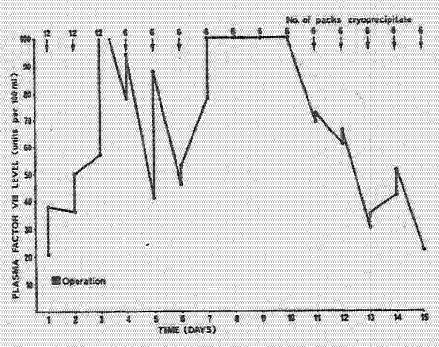


Fig. 4. Case 2. Plasma Factor VIII levels produced by cryoprecipitate infusions over a 15-day period following nasal mucosal resection. Each arrow indicates a separate infusion and the number of packs infused.

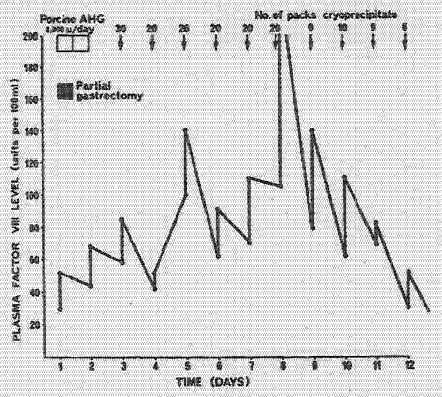


Fig. 5. Case 3. Plasma Factor VIII levels produced by porcine AHG and cryoprecipitate infusions over a 15-day period following partial gastrectomy. Each arrow indicates a separate infusion and the number of packs infused.

Elective surgery has been undertaken in 10 patients. Of these 4 had major procedures: one dental clearance, one E.N.T. operation and 2 abdominal operations. The case reports of these 4 patients are now given in detail.

Case 1. This 35-year-old haemophiliac had sustained many spontaneous joint and muscle bleeds in the past and had bled profusely after previous tooth extractions. He has clinically severe haemophilia (plasma factor VIII level = 0 units/100 ml. *i.e.*, no assayable factor VIII level). On this occasion 10 teeth were extracted under cryoprecipitate cover. The sockets were packed with 'Surgicel' and a preconstructed splint inserted. At operation there was no excess bleeding and in the next 48 hours only slight flecks of blood appeared in the saliva. Thereafter healing was uneventful, there being no further bleeding and the patient was discharged

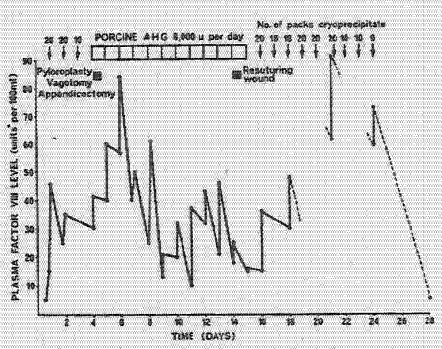


Fig. 6. Case 4. Plasma Factor VIII levels produced by cryoprecipitate and porcine AHG over a 26-day period following pyloroplasty, vagotomy and appendicectomy. Each arrow indicates a separate infusion and the number of packs infused.

home on the 14th post-operative day. The results of his factor VIII assays are shown in Figure 3. A total of 215 packs of cryoprecipitate was used.

Case 2. This patient presented with a 30-year history of bleeding only after surgical procedures. He has mild haemophilia (factor VIII level=18 units/100 ml.). He had had 9 previous nasal operations each of which was complicated by excess bleeding and necessitated blood transfusion and prolonged stay in hospital. He presented with a large mucocoele of the frontal air sinuses. At operation it was found to be extending laterally into both orbits and posteriorly into the ethmoid air sinus. During dissection there was some extravasation of blood into both orbits accompanied by some excess bleeding. Therapy was continued for 14 days. Post-operatively no bleeding was recorded and healing was uneventful. The results are shown in Figure 4. Ninety-six packs of cryoprecipitate were given.

Case 3. This patient presented with dyspepsia, weight loss, and epigastric pain radiating through to his back. Barium meal showed the presence of both a gastric and a duodenal ulcer. He has moderate haemophilia (factor VIII level = 5 units/100 ml.). Billroth type I partial gastrectomy and vagotomy were undertaken using porcine factor VIII. On the third day the patient had an anaphylactoid reaction with rigors and hypotension while being given the porcine preparation, which was discontinued. Haemostasis was maintained using cryoprecipitate which was given for 12 days. The post-operative course was uneventful. The results are shown in Figure 5. A total of 180 packs of cryoprecipitate was used.

Case 4. This patient had had several episodes of haematemesis and melaena over the previous 13 years and was known to have a duodenal ulcer. He had a moderate degree of haemophilia (factor VIII level = 5 units/100 ml.). On this occasion he was admitted with further melaena and treated with cryoprecipitate. Pyloroplasty and vagotomy were carried out under cover of porcine factor VIII and the opportunity was taken at the same time to perform an elective appendicectomy. The post-operative course was stormy being complicated by a 'paralytic' ileus. By the 12th day post-operatively the patient had developed resistance to the porcine

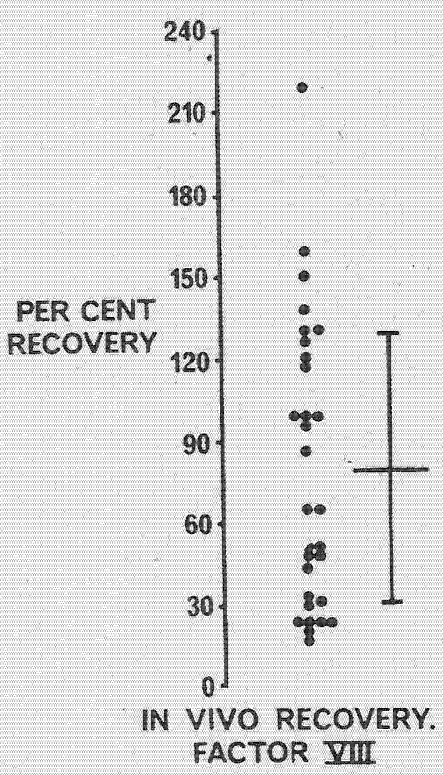


Fig. 7. Percentage Factor VIII recovery *in vivo* on 30 occasions following cryoprecipitate therapy showing the mean (80 per cent) ± 1 standard deviation (79 per cent).

material with the result that the factor VIII level did not rise after infusion of as much as 8,000 units. At this stage the abdominal wound dehisced. Resuturing was undertaken covered by cryoprecipitate. Therapy was continued for a further 10 days until the wound was fully healed. The results are shown in Figure 6. A total of 168 packs of cryoprecipitate was used.

RESULTS

In vitro assays of cryoprecipitate preparations

The results of factor VIII assays of 46 separate packs of cryoprecipitate sampled just before pooling are shown in Figure 2. The values are expressed as units of factor VIII per pack. The average was 130±79 units per pack.

Recovery *in vivo* of cryoprecipitate (factor VIII)

This was calculated on 30 occasions and the results are shown in Figure 7. The average recovery of infused factor VIII was 80 per cent of that expected.

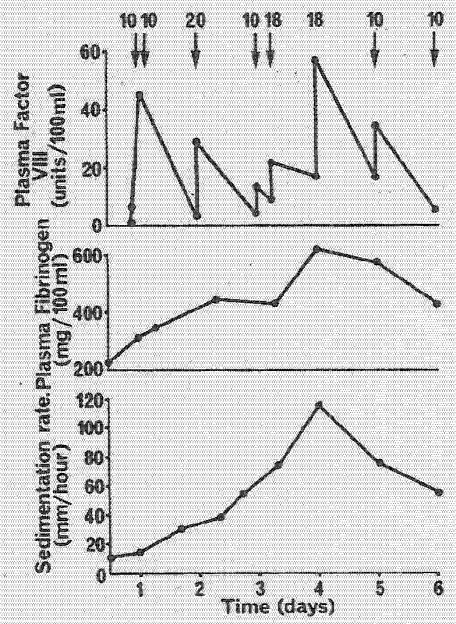


Fig. 8. Plasma Factor VIII and fibrinogen levels and erythrocyte sedimentation values in a patient treated with cryoprecipitate for 6 days. Each arrow indicates a separate infusion and the number of pack infused.

Half-life of infused cryoprecipitate (factor VIII)

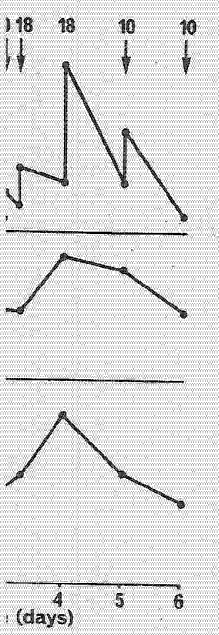
The results of 36 estimations show that the half-life of factor VIII administered as cryoprecipitate is 10±2 hours. These figures agree with those of Douglas (1958), Biggs and Denson (1963) and Abildgaard *et al.* (1964) for other preparations of factor VIII.

Survival of factor VIII *in vivo*

This was calculated from assay results obtained immediately after a dose and immediately before the next dose 12 or 24 hours later. The results were calculated from 36 infusions.

Fibrinogen concentration

Cryoprecipitate is rich in fibrinogen. Brown *et al.* (1967) found that the concentration of clottable protein in their cryoprecipitate preparation was 11 times as high as compared with the plasma from which it was derived. The average concentration of fibrinogen in the cryoprecipitate described in this paper was 557±21 mg. per 100 ml., *i.e.* only about



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2 to 3 times that of plasma. During a course of treatment with cryoprecipitate there is a progressive increase in the plasma fibrinogen level in the recipient, the half-life of fibrinogen being considerably greater than that of factor VIII. No side effects were found arising from the elevated fibrinogen level. However, as might be anticipated erythrocyte sedimentation rate rose in parallel with fibrinogen level (Fig. 8).

Red Cell antibodies

No red cell antibodies were found.

Sterility

No bacterial contamination was found on culture of the content of 250 bags.

Factor VIII inhibitors

Cryoprecipitate was used in two patients who had factor VIII inhibitors.

Case 5. This patient was transferred to our care because of prolonged bleeding from the intestinal tract. He had been treated in the past with whole blood and fresh frozen plasma. After infusion of cryoprecipitate (20 packs) no factor VIII activity was recovered from his plasma though the expected rise in the plasma factor VIII level was 19.2 units per 100 ml. plasma. Further examination of the plasma revealed that the inhibitor was active against human, porcine and bovine factor VIII. Despite massive dosage of factor VIII concentrate and blood transfusion the patient died of continued intestinal bleeding.

Case 6. This patient was a mentally retarded child with clinically severe haemophilia. He had received many infusions of plasma in the past, the infusion usually having been given by a venous 'cut-down'. In the past 4 years no therapy had been given because of the difficulty in venepuncture. On this occasion he had severe haematuria which resulted in a rapid fall in his haemoglobin. Cryoprecipitate therapy was given on 3 days in a high dosage but no clinical improvement resulted. No assays were performed because of the difficulty in obtaining venous blood. Further examination of his blood showed the presence of a potent inhibitor against human, bovine and porcine factor VIII. Treatment was started with aminocaproic acid and bleeding ceased. The haematuria has not recurred over a period of 8 months during which time he has continued to receive the fibrinolytic inhibitor.

Hepatitis

During this study 2 of our patients developed jaundice after cryoprecipitate therapy. Both however had received, in addition, blood or fresh frozen plasma.

Case 4. This patient had many previous admissions for haematemesis and melaena. He was re-admitted with severe melaena, transfused 6 pints of whole blood and was given a 7-day course of cryoprecipitate during which he received 95 packs. He became jaundiced 36 days after this therapy had been given and was attended by his general practitioner at home. Two weeks later liver-function tests showed evidence of hepatocellular damage. SGPT = 340 SK units per ml.; SGOT = 150 SK units per ml.; total bilirubin = 2 mg. per 100 ml., direct reacting = 1.8 mg. per 100 ml.; thymol turbidity = 1.8 units; alkaline phosphatase = 14 KA units. Within a few days symptoms and signs had disappeared and the liver function tests returned to normal, and remained so over the next few weeks.

The patient was re-admitted 12 weeks later with a recurrence of his melaena, treated once again with cryoglobulin precipitate and a pyloroplasty/vagotomy and appendicectomy were carried out under cover of porcine AHG. The post-operative course of the patient has been described above. Routine follow up showed biochemical evidence of recurrence of hepatitis six weeks after the second course of cryoprecipitate. Liver function tests: SGPT = 420 SK units/ml.; SGOT = 430 SK units per ml.; bilirubin 1.3 mg per 100 ml., direct bilirubin 0.5 mg. per 100 ml. These results returned to normal over the course of 2 months, and the patient has been symptomless since.

Case 7. This moderately affected haemophiliac had been treated for haematuria with fresh frozen plasma (1 litre) but had continued to bleed. He was then transferred to the Regional Haemophilia Centre and given a 4-day course of cryoprecipitate, receiving 48 packs in all. Five months later he presented with a story of recent nausea and vomiting; he was mildly jaundiced: Liver function tests SGPT = 650 SK units per ml.; SGOT = 550 SK units per ml.; total bilirubin = 3.2 mg. per 100 ml.; direct bilirubin = 2.3 mg. per 100 ml.; thymol turbidity 3.1 units.

DISCUSSION

Our results confirm those of Pool and Shannon (1965), Djerassi *et al* (1965), Hattersley (1966), Prentice *et al.* (1967), Bennett *et al* (1967) and Brown *et al* (1967) that cryoprecipitate is an effective therapeutic material in the treatment of bleeding episodes in the haemophiliac. It contains small amounts of other coagulation factors (see Table III) but has no therapeutic usefulness in any other bleeding state, except von Willebrand's disease. The advantages and disadvantages of this material are shown in Table IV.

The complications noted are largely those which result from any infusion of a fibrinogen containing fraction. Two of our patients developed jaundice after receiving cryoprecipitate but they had also received blood and

Table III. Cryoprecipitate—constituents.
Mean and 1 standard deviation of 10 samples.

Factor	
II	48 ± 19%
V	65 ± 9%
IX	52 ± 6%
VIII	130 ± 79% c.u.
Fibrinogen	557 ± 21 mg per 100 ml.
Plasminogen	2.8 ± 0.25 c.u./ml.
Plasminogen activator	Nil

Table IV. Advantages and disadvantages of cryoprecipitate therapy.

Advantages
Simply prepared, does not require costly apparatus. Can be made by the local blood transfusion service.
Stable on storage at —70°C. and the material is free from bacterial contamination.
Administered as a single quick infusion or injection in small volume and so does not cause circulatory overload.
No wastage of the red cells or of the supernatant plasma.
Does not stimulate production of red cell antibodies.
Disadvantages
(in common with other sources of factor VIII)
May transmit hepatitis virus.
May induce factor VIII inhibitor.
Variation in patients makes standardisation of dosage difficult.

fresh frozen plasma. In one patient there was biochemical evidence of recurrence of hepatitis after a second course of therapy. Hepatitis after cryoprecipitate therapy has been reported before by Besselaar *et al.* (1966) and Del Duca (1966) but as yet there is no proof that the incidence of this complication is any higher than after other types of plasma therapy. In theory one would expect a higher incidence of hepatitis due to the large number of donors used to prepare one cryoprecipitate dose.

Despite follow-up for a period of 6 months, no evidence was found in our patients that this material stimulated production of factor VIII inhibitors even though several of the patients have had multiple courses of treatment. This complication has, however, been described in patients by Hattersley (1966) and Besselaar (1966).

Minor side effects—slight rigors, urticaria, tingling of the tongue—were relatively common. Two patients developed sudden severe pain in the lumbar region, a complication apparently related to the speed of infusion; the pain disappeared as the rate of infusion was reduced but returned when the rate was increased.

The evidence suggests that cryoprecipitate represents an important advance in the therapy of haemophilia. However, in view of the somewhat variable levels of factor VIII, achieved *in vivo* with cryoprecipitate, further clinical investigation is needed before it can be recommended for routine use without laboratory control.

The availability of cryoprecipitate also offers the possibility of assessing the management of haemophilia in childhood using well designed trials; for example the role of this material in the possible prevention of haemarthrosis requires to be examined.

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