Antihaemophilic Globulin: Preparation by an Improved Cryoprecipitation Method and Clinical Use

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The control of bleeding episodes in haemophilia and the prevention of excessive haemorrhage after surgical procedures depend chiefly on raising the patient's plasma factor VIII concentration to an appropriate haemostatic level, and maintaining this so long as the likelihood of bleeding continues. With whole plasma it is impossible to achieve levels much above 25%, or to maintain values constantly above about 10% for more than two or three days, without overloading the patient's circulation. In order to overcome this problem antihaemophilic globulin concentrates have been prepared for clinical use by a variety of methods. Most of these require much time and technical skill, and involve considerable loss of activity during the process. The cryoprecipitate method of Pool and her associates, however (Pool *et al.*, 1964; Pool and Shannon, 1965), based on their observation (Pool and Robinson, 1959) that when frozen plasma is thawed in the cold most of the factor VIII remains in the cold-insoluble precipitate, provides a simple means of preparation of potent antihaemophilic globulin concentrates, suitable for introduction as a routine blood-banking procedure.

We report here 12 months' experience in the preparation and clinical use of cryoprecipitate antihaemophilic globulin, and describe a modification of Pool's original method of preparation, which has resulted in a great improvement of recovery of factor VIII activity in the concentrates.

Preparation of Antihaemophilic Globulin Concentrate

Slow Method (Pool and Shannon, 1965)

Approximately 500 ml. of blood was taken from each donor into a double pack (Fenwal JD-2) by a clean venepuncture. Continuous gentle agitation ensured thorough mixing of the blood and the acid-citrate-dextrose anticoagulant throughout the bleeding. At the end of the blood donation 5 ml. of well-mixed blood was run back out of the pack for factor VIII assay. The blood pack and assay sample were then rapidly cooled to 2-4° C. in a water-bath containing melting crushed ice, and kept at this temperature until they could be transferred to a precooled centrifuge at 4° C. The pack was then centrifuged for 30 minutes at 1,600 *g* to obtain the maximum yield of platelet-poor plasma, while the assay sample was centrifuged and assayed immediately for factor VIII.

Not more than 280 ml. of plasma, free of red cells and buffy coat, was transferred to the 300-ml. satellite pack, which was placed in a cardboard carton and immersed in a mixture of solid CO₂ and ethanol at about -70° C. in a large vacuum flask, leaving the attached main blood pack on the bench beside it. The plasma was allowed to remain in the freezing mixture for at least 10 minutes, and then both packs, still joined

together, were placed in a 4° C. refrigerator and allowed to thaw for 18 to 24 hours. As soon as thawing was completed, both packs were centrifuged for 20 minutes at 4° C. and 1,600 *g*. After centrifugation the satellite pack was gently inverted about 18 in. (46 cm.) above the main blood pack and the supernatant plasma was run back by gravity on to the cells to reconstitute the blood. The packs were separated and a small sample of supernatant was kept for factor VIII assay. The cryoprecipitate remained behind, adhering to the walls of the satellite pack, and was immediately reconstituted for use or stored at -30° C. until required. During the earlier part of the study cryoprecipitate was reconstituted by adding 5-15 ml. of saline to the pack and gently agitating in a 37° C. water-bath for about two minutes to redissolve the precipitate. Later, 5-15 ml. of supernatant was left in the pack for this purpose.

Rapid Method

Blood was taken into 500-ml. single packs (Fenwal JA-2C), which were handled and centrifuged as in the slow method, described above, to obtain platelet-poor plasma. As much plasma as possible (260-340 ml.) was then transferred to a 600-ml. transfer pack (Fenwal TA-1), which was flattened between two sheets of copper mesh and frozen at about -70° C. In this way, plasma froze as a thin slab more rapidly than in the 300-ml. transfer pack used in the slow method.

The plasma was allowed to remain in the freezing mixture for six minutes, and was then transferred to a water-bath at 6-8° C. As soon as the outer layer of plasma began to thaw, the pack was gently kneaded to enable thawing to continue as evenly and rapidly as possible throughout the pack. This process ensured complete thawing of the plasma within 90 minutes. The pack was then centrifuged at 1,600 *g* for 15 to 20 minutes, and the supernatant plasma removed, leaving 5-15 ml. in the pack with the cryoprecipitate. As double-pack units with attached 600-ml. transfer packs were not available, this was not a closed system and so the plasma was not returned to the red cells.

If not used immediately the cryoprecipitate was snap-frozen at -70° C. before storing at -30° C. until use.

Administration of Concentrate

All concentrates were administered intravenously by syringe within half an hour of reconstitution, not more than about five minutes being taken for each dose. From a vein distant from the site of injection, blood samples for assay were taken into one-ninth volume of acid-citrate-dextrose immediately before injection and again 15 to 20 minutes afterwards. In small infants capillary samples were sometimes used.

Patients received cryoprecipitate of the same ABO and rhesus groups except towards the end of this work, when a few patients were given cryoprecipitate of heterologous ABO groups but homologous rhesus groups.

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Factor VIII Assays

Factor VIII assays were performed on the original donor plasma, the supernatant plasma after separation from the cryoprecipitate, the redissolved cryoprecipitate, and the samples obtained from the patient before and after injection of concentrate. The assays were carried out by the kaolin-activated one-stage method of Hardisty and Macpherson (1962) with the modification that the inosithin was added after the preincubation with kaolin.

A pool of four normal plasmas, from blood collected into one-ninth volume of acid-citrate-dextrose, was used as the control for each test. This was snap-frozen in 0.2-ml. aliquots at -30°C . and renewed fortnightly, so far as possible from the same donor pool. Good agreement in potency was obtained between this control and a freshly reconstituted freeze-dried antihaemophilic-factor reference plasma (Hyland 35-811) used within one hour of reconstitution at 4°C .

Cryoprecipitate concentrates were diluted 1 in 10 to 1 in 30 before assay, in order to reduce their activity to approximately that of whole plasma. In the early part of the study this initial dilution was carried out in veronal-buffered saline; later, supernatant plasma was used as diluent, in a proportion calculated to restore the activity of the concentrate to approximately that of the plasma from which it was derived. The final result of the assay in this latter system was corrected for the activity contributed by the supernatant diluent. The following is an example of the calculations employed:

Original fresh plasma volume = 280 ml
Reconstituted cryoprecipitate volume = 14 ml
Original fresh plasma assay = 100%
Assay of 1 part of cryoprecipitate diluted in 19 parts of supernatant plasma = 90%
Supernatant assay ... = 15%
 $\therefore$ true assay of concentrate = $(90 \times 20) - (15 \times 19)$
= 1,800 - 285
= 1,515%

Calculation of Factor VIII Recoveries

One unit of factor VIII is defined as the activity of 1 ml. of plasma with an assay value of 100%. In order to calculate the recovery of factor VIII at the various stages of preparation, the total activities of the original donor plasma, the supernatant plasma after freezing and thawing, and the redissolved concentrate were calculated from the formula:

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

The residual activity remaining in the supernatant plasma was expressed as a percentage of that in the original donor plasma, and the recovery in the concentrate as a percentage of the starting activity (original plasma—supernatant).

The in-vivo recovery of activity after infusion was expressed as a percentage both of the activity of the concentrate and of that of the starting material (original plasma—supernatant), the following formula being used for the calculation of the activity recovered in the patient's plasma:

$$\frac{\text{Factor VIII recovered (units)}}{\text{Plasma volume (ml.)} \times \text{factor VIII rise (\%)}} \times 100$$

Plasma volumes were estimated from the venous haematocrit and body weight, the data given by Mollison (1962) being used.

Total protein was estimated by a micromodification of the biuret method of King and Wootton (1956).

The clottable protein in 5 ml. of plasma or concentrate was estimated by the clot-weight method of Ingram (1952).

Results

Factor VIII Recoveries in vivo and in vitro

Of 278 packs of cryoprecipitate prepared by the slow method, 221 were administered to young haemophiliacs as 75 separate doses; 103 packs of cryoprecipitate were prepared by the rapid method and injected as 61 separate doses. In-vivo recoveries of activity were determined after 105 doses—51 of material prepared by the slow method and 54 by the rapid method. The mean in-vitro and in-vivo recoveries of both types of material are shown in Table I. Fewer in-vivo than in-vitro recoveries were available, as individual doses often consisted of more than one pack.

TABLE I.—Factor VIII Recoveries

	Slow Method		Rapid Method	
	No. of Estimations	Recovery (%) (Mean $\pm$ S.D.)	No. of Estimations	Recovery (%) (Mean $\pm$ S.D.)
Remaining in supernatant plasma	278	16.0 $\pm$ 8.0	103	21.6 $\pm$ 6.2
In concentrate*	221	48.7 $\pm$ 31.7	103	37.0 $\pm$ 23.1
In patient's plasma after injection*	51	50.8 $\pm$ 17.3	38	79.0 $\pm$ 17.8
In patient's plasma after injection†	50	111.7 $\pm$ 46.4	54	90.7 $\pm$ 21.6

* As a percentage of the activity of the original plasma, less that of the supernatant.
† As a percentage of the activity of the concentrate.

Concentrates prepared by the slow method were assayed after initial dilution in buffered saline, and those prepared by the rapid method after dilution in supernatant plasma (see Methods).

It can be seen that when cryoprecipitate was prepared by the slow method about 16% of the original activity was left in the supernatant fraction; just over half of the remaining 84%, or about 43% of the original activity, was recoverable in the patient's circulation after injection. It follows that an average of 41% of the original activity was lost during the processing or after administration.

The corresponding figures for the rapid method show that 23% of the original activity was left in the supernatant, and 79% of the remainder, or 61% of the original activity, could be recovered after injection. Thus only 16% was lost during processing and administration, as compared with 41% by the slow method. Since there was no reason to suppose that material prepared by the two methods would behave differently in vivo, it seemed likely that this difference was attributable solely to better conservation of activity during preparation by the rapid method.

The data in the second and fourth rows of Table I (in-vitro recovery of factor VIII activity in the concentrates, and in-vivo recovery relative to the concentrate activity) depend on the results of factor VIII assays on the concentrated material. During the first part of the study, while cryoprecipitate was being prepared by the slow method, these assays were being performed on concentrates after initial dilution in buffered saline (see Methods). It can be seen that there was a wide scatter of results and a tendency for apparent in-vivo recoveries of over 100% to be obtained, in association with relatively low in-vitro recoveries. A contributory cause of these findings was the inherent inaccuracy of an assay procedure designed for the measurement of factor VIII concentrations of the order of those found in normal plasma, when applied to the assay of highly concentrated material; at the high dilutions necessary in the system, small differences in clotting-times reflected large differences in activity of the concentrates.

A modified technique for assaying the concentrates was therefore developed (see Methods) in an attempt to overcome these difficulties; this method, involving an initial dilution of concentrate in supernatant plasma, was introduced at the same time as the rapid method of preparation. It is apparent from the last column of Table I that the scatter of results was much narrower than with the previous method, and that a much higher mean in-vitro recovery was associated with a mean

in-vivo recovery of rather below 100%. The slight diminution in mean in-vivo recovery was probably due to correction of the tendency of the original assay system to underestimate the activity in concentrates. Such an explanation would have accounted for only about one-quarter of the difference between the mean in-vitro recoveries in concentrates prepared by the two methods, and the remaining difference must be attributable to a true increase in yield achieved by the rapid method. This conclusion is supported by the difference in overall in-vivo recoveries relative to the activity of the original plasma, as shown in the third line of Table I.

Direct confirmation of the increased in-vitro yield attainable by the rapid method was obtained from the following experiment. Pools of two donor units of plasma (500–600 ml.) were assayed and then divided into two equal portions and frozen at -70°C . One was thawed in air at 4°C . (16–24 hours) and the other in a $6-8^{\circ}\text{C}$. water bath (about 90 minutes), the cryoprecipitate being harvested in each case as soon as complete thawing had occurred. Both cryoprecipitates were then redissolved in a small volume of supernatant plasma and assayed by the modified technique. Seven such comparisons were made and the in-vitro recovery of factor VIII in the material prepared by the rapid method was higher in every case (Fig. 1), the mean values being 84% by the rapid method and 49% by the slow method. These figures are in close agreement with those shown in Table I for the whole series of concentrates.

FIG. 1.—Recovery of factor VIII in cryoprecipitate after slow and rapid thawing of duplicate plasma samples.

Total Protein and Fibrinogen Concentration

Total and clottable protein were measured in 10 samples of cryoprecipitate prepared by the rapid method, redissolved in a few millilitres of supernatant plasma and assayed for factor VIII by the modified technique (see Methods). The results of these estimations are compared with average values for whole normal plasma in Table II. The mean total protein concentration in the redissolved cryoprecipitates is only slightly higher than that

TABLE II.—Protein and Factor VIII Concentration in Cryoprecipitates

	Volume (ml.)	Protein		Factor VIII		
		Total (g./100 ml.)	Clot-table (% Total)	Concentration (%)	Total Units per Plasma Pack	Units per g. Protein
Concentrate (10 samples):						
Mean ..	12.2	7.8	55	2.265	267	289
Range ..	9–17	5.7–8.7	28–84	1,589–3,695	194–369	218–445
Normal plasma (average values)	320	7.0	5	100	320	14

of normal plasma, though the proportion of clottable protein is 11 times as high. The mean factor VIII concentration in redissolved cryoprecipitates is thus about 23 times that of normal plasma in terms of volume, and over 20 times that of plasma in terms of protein concentration. By redissolving the cryoprecipitate in the smallest possible volume of supernatant plasma, or in sterile saline, still higher concentrations can be achieved; in practice, however, this is unnecessary.

Each pack of concentrate contained about 0.5 g. of fibrinogen (range 0.23–0.62 g.); at the usual dosage for minor bleeding episodes (20–25 units of factor VIII per kg.), a single dose of concentrate would be expected to raise the recipient's plasma fibrinogen concentration by about 60–80 mg./100 ml. Because the half-life of fibrinogen in the circulation is much longer than that of factor VIII, the effect on plasma fibrinogen concentration of a course of injections was cumulative.

Fibrinogen concentrations were measured on 22 occasions after injection of one or more doses of concentrate; the values ranged between 450 and 1,150 mg./100 ml. The highest concentration was observed in a boy who had received three doses on successive days; it fell gradually during the subsequent 48 hours, and produced no side-effects.

Simultaneous determinations of factor VIII and fibrinogen concentrations in vivo were made after four successive injections of concentrate, derived from a total of 18 packs of cryoprecipitate, into a 10-year-old boy to cover the correction of a flexion deformity of the knee. The results are shown in Fig. 2, from which it can be seen that the patient's plasma fibrinogen concentration rose by about 100 mg./100 ml. after each dose of concentrate, reaching 735 mg./100 ml. after the last dose.

FIG. 2.—Fibrinogen and factor VIII concentrations after repeated injections of antihæmophilic globulin concentrate (indicated by arrows) in a boy aged 10 weighing 26 kg.

In-vivo Survival of Factor VIII

The mean half-disappearance time of factor VIII after 27 injections in 15 patients was 8.5 hours (range 6.0–13.5 hours). These values were derived from assay results obtained immediately after a dose and immediately before the subsequent dose, 12 to 24 hours later. More numerous assays were performed after six injections of concentrate in three patients, none of

whom was bleeding at the time; the in-vivo survival curves for factor VIII in these cases are shown in Fig. 3. All but one of these curves are biphasic, as has been observed by many others who have studied the in-vivo survival of factor VIII. The initial rapid phase showed a mean half-disappearance time of 5.5 hours (range 4.3–6.0 hours), and the slower second component represented a mean biological half-life of 10.8 hours (range 6–14 hours). These results agreed fairly closely with those of other workers, using a variety of preparations containing factor VIII—for example, Shulman *et al.* (1962), Adelson *et al.* (1963), Biggs and Denson (1963), and Abildgaard *et al.* (1964).

FIG. 3.—Survival of factor VIII in vivo after injection of concentrate.

Storage Stability

Concentrate was stored at -30°C . for periods up to 17 weeks before use, but the demand for the material was such that nearly all was used within 10 weeks of preparation. In Fig. 4 the in-vitro recovery of factor VIII activity in individual packs of concentrate at the time of use, as a percentage of that of the original donor plasma, is related to the duration of storage. It appears that no significant loss of activity occurred over a period of at least 10 weeks.

FIG. 4.—In-vitro recovery of factor VIII in cryoprecipitates stored for various periods at -30°C .

Clinical Results

Fifty-nine episodes requiring treatment with 161 separate doses of antihaemophilic globulin concentrate occurred in 23 young haemophiliacs aged from 16 days to 14 years. Twenty-five doses, including 18 used for the treatment of one major surgical case (Case 2), were supplied by the North-West Metropolitan Regional Blood Transfusion Centre, and the remainder were prepared by us.

Antihaemophilic globulin concentrate was given as specific treatment for spontaneous and traumatic bleeding episodes, as cover for various dental procedures and orthopaedic manipulations and for two life-saving surgical operations. The dosages and laboratory results are summarized in Table III. The dose of antihaemophilic globulin concentrate to be administered was calculated from the assay result on the original donor plasma from which it was derived, in terms of the patient's estimated plasma volume and the plasma factor VIII concentration required for the adequate treatment of the particular lesion, thus:

$$\text{Total factor VIII required (units)} = \frac{\text{Intended rise in plasma factor VIII (\%)} \times \text{plasma vol. (ml.)} \times 2}{100}$$

The factor of 2 is included because the in-vivo recovery of activity, relative to that of the donor plasma from which the concentrate was derived, was of the order of 50% (see above). Since the plasma volume usually falls within the range of 40–50 ml./kg. body weight, it follows that a dose of factor VIII of 1 unit/kg. would be expected to raise the patient's factor VIII concentration by a little over 1%, again allowing for 50% recovery. Though this useful working rule was not used in the calculation of dosage, its validity was borne out by many of our results (Table III).

TABLE III.—Cryoprecipitate Antihaemophilic Globulin: Clinical Results

Lesion	No. of Episodes Treated	Total Doses Given	Mean Dose* (units/kg.)	Post-infusion Factor VIII	
				Mean (%)	Range (%)
Haemarthroses	25	31	23	29	10–55
Orthopaedic procedures ..	12	33	23	29	18–46
Haematomata	11	22	24	25	12–50
Lacerations	2	3	40	59	42–76
Dental procedures	6	19	20	29	15–38
Haematuria	1	4	14	39	27–47
Intracranial haemorrhage ..	1	31	37	66	40–100
Strangulated hernia ..	1	18	100 (approx.)	105	34–183

* Based on assay of original fresh plasma.

Haemarthroses

Twenty-five acute haemarthroses were treated in 12 patients with a total of 31 doses of antihaemophilic globulin concentrate. Parents were encouraged to ask for treatment for their sons as soon as it was apparent to them that a haemarthrosis impairing mobility or causing pain or swelling was developing. A single dose of concentrate was given immediately after hospital admission or, in the less severe cases, while the patient attended the outpatient department. In three patients with haemarthrosis of the knee, aspiration was performed immediately after injection of concentrate.

On 14 occasions rapid clinical improvement, as assessed by loss of pain, swelling, and limitation of movement, occurred within 24 hours and only one dose of concentrate was required. There was no recurrence of bleeding, and the mean factor VIII concentration after injection was 33%. On 11 other occasions clinical improvement was delayed for 24 to 48 hours, and five of these cases required additional doses of concentrate on successive days before clinical improvement occurred. The mean post-injection factor VIII concentration for these cases was 24%.

Three haemarthroses were treated on an outpatient basis, and in 12 other instances the patient was discharged from hospital within 48 hours of admission; in most of the remaining 10 instances the patient was kept in hospital for up to four days, to allow gradual mobilization of the joint under supervision.

Orthopaedic Procedures

Five patients required antihaemophilic globulin concentrate as cover for a variety of orthopaedic manoeuvres, involving 12 separate episodes of treatment (Table III). One 13-year-old patient required 15 daily doses of concentrate, in three courses of five days each, for the fitting and extension of a "wedging-plaster" to correct a long-standing flexion deformity of the knee. No bleeding occurred during this period of treatment, and the mean post-injection factor VIII concentration attained was 31% (range 21–41%). A second patient (aged 14) required eight doses of concentrate to cover physiotherapy after aspiration of a chronic effusion in the knee joint. Reabsorption of the remaining fluid was slow and full stability of the joint was not achieved until eight weeks later. Both these patients had radiological evidence of early degenerative changes in the affected knee at the time of treatment. Another patient required cover for physiotherapy after joint aspiration, and the remaining two patients were each given one dose of concentrate to cover the early stages of ambulation with walking-splints. A post-injection factor VIII concentration of 30% was aimed for in these cases and was usually achieved; none suffered any bleeding as a result of these procedures.

Haematomas and Lacerations

Eleven episodes of single or multiple haematomas severe enough to require treatment with antihaemophilic globulin concentrate occurred in six patients (Table III). Nine of these episodes were uncomplicated haemorrhages into limb-girdle or limb muscles—for example, deltoid, pectoralis major, calf, thigh—and rapid clinical improvement, shown by disappearance of pain and swelling and return of movement, occurred after one dose of concentrate. Two patients presented with iliacus haematomas complicated by femoral-nerve palsy due to pressure on the nerve in the tense iliacus sheath. The first case was treated with five daily doses of concentrate to a mean peak factor VIII concentration of 26%, and the palpable haematoma in the right iliac fossa disappeared. The second patient maintained clinical improvement after only three courses, each of three daily doses of concentrate, spread over a period of four weeks. This patient developed a weak factor VIII inhibitor, and is discussed below. Treatment was stopped in both patients as soon as it was apparent that the haematoma was being absorbed. The femoral-nerve lesion gradually recovered in each case, with full recovery of power and sensation in the affected thigh after two and a half and four months respectively.

Two patients, aged 2½ and 4, with lacerations of the tongue and frenulum respectively, were treated with relatively high doses of concentrate (Table III) as these lesions are potentially hazardous and impossible to immobilize satisfactorily, particularly in young children. Bleeding stopped after the first dose in each case, and did not recur. The elder child received a second dose 24 hours later.

Dental Procedures

Four patients required extractions of deciduous teeth, successful haemostasis being maintained with three to five successive daily doses of concentrate, giving mean peak factor VIII concentrations of 28–38%. Two other patients were treated with somewhat lower doses for haemorrhage around loose deciduous teeth.

Haematuria

A 14-year-old boy developed severe spontaneous haematuria and clot colic. In spite of a peak factor VIII concentration of 47% after the first dose of cryoprecipitate, haematuria recurred after 24 hours, and three further days' treatment to levels of 27–46% were required to achieve lasting haemostasis.

Major Surgery

Two small boys required major surgery on account of intracranial haemorrhage and strangulated hernia respectively. Haemostasis was satisfactorily maintained with cryoprecipitate in each case, as described below.

Case 1 (Fig. 5).—A 2-year-old-boy was admitted to hospital one month after having fallen downstairs, with the clinical features of a slowly progressive intracranial haemorrhage. His condition deteriorated during the next three days, despite daily doses of cryoprecipitate antihaemophilic globulin; a carotid arteriogram performed under antihaemophilic globulin cover showed evidence of a left subdural haematoma extending across the floor of the middle fossa. Craniotomy was therefore performed, and the haematoma evacuated, a rubber drain being left in position for 24 hours. Cryoprecipitate antihaemophilic globulin from three packs, given immediately before operation, raised his factor VIII to over 90%; it was subsequently maintained at over 30% for the next seven days, and over 20% for the second postoperative week, by two or three daily doses administered via a continuous intravenous infusion. There was no excessive bleeding at operation or subsequent blood loss; no red cells were transfused. In all, this patient, whose blood volume was about 750 ml., received antihaemophilic globulin derived from 16 litres of plasma within 14 days, in a total volume of 890 ml. Recovery was complete, with no residual neurological effects.

FIG. 5.—Case 1. Plasma factor VIII concentrations during replacement therapy with antihaemophilic globulin concentrate.

Case 2.—A premature infant (birth weight 2.1 kg.) was admitted at the age of 16 days on account of acute small-bowel obstruction, apparently due to a strangulated inguinal hernia. As two of his mother's brothers were known to have haemophilia, blood had been taken in another hospital by femoral vein puncture for coagulation tests, and a large haematoma below the inguinal ligament had resulted. He also had a ventricular septal defect of moderate size. Laparotomy, performed after administration of cryoprecipitate antihaemophilic globulin derived from 1 pint (570 ml.) of blood (which raised the patient's factor VIII to 137%), revealed a Meckel's diverticulum incarcerated in the right inguinal canal. The diverticulum was resected and the bowel joined by an end-to-end anastomosis, and an inguinal herniotomy was performed with removal of the sac. There was no excessive bleeding at operation. He was digitalized, and replacement therapy was continued with cryoprecipitate antihaemophilic globulin at approximately 12-hourly intervals for the next nine days, when he developed congestive cardiac failure with pulmonary oedema; transfusion was therefore stopped, and he recovered. A single further dose of cryoprecipitate was given five days later for the removal of the remaining sutures, which had been left in place on account of a wound infection. His

convalescence was uneventful, and there was no evidence of bleeding at any time. During his whole course he received no red cells, but was given antihaemophilic globulin derived from about five litres of plasma, in a total volume of about 250 ml. His own blood volume on admission was about 230 ml.

Factor VIII Inhibitors

Factor VIII inhibitors were found in two patients. The first was seen with a bleeding tooth socket from a loose deciduous tooth while he was visiting London. After injection of concentrate on two successive days, no factor VIII activity could be recovered *in vivo*, and further investigation showed that his plasma contained a potent factor VIII inhibitor; at a dilution of 1:512 this was capable of destroying 50% of the factor VIII activity of fresh plasma in 10 minutes at 37° C. This inhibitor had apparently developed while the patient was receiving whole-plasma therapy; he had not previously been treated with concentrated antihaemophilic globulin.

The second patient developed resistance to treatment during a four-week period in which he received concentrate in three short courses for an iliacus haematoma. Fig. 6 shows the failure of increasing doses of concentrate on days 27–29 to raise the *in-vivo* factor VIII concentration to expected values.

FIG. 6.—Development of resistance to treatment with antihaemophilic globulin concentrate in a boy aged 7½ years. The open columns represent the dose of concentrate and the black columns the patient's plasma factor VIII concentration immediately after each dose.

On day 30, after cessation of treatment, his plasma showed a weak factor VIII inhibitor capable of destroying 50% of the factor VIII activity of fresh plasma in 30 minutes at 37° C. in a 1:1 mixture. This inhibitor was still present at the same titre three weeks later.

Side-effects and Reactions

No early or late generalized hypersensitivity reactions occurred, even in three patients who had required corticosteroid or antihistamine cover for previous whole-plasma transfusions, and there were no local reactions at the site of injection.

No cases of serum hepatitis have been reported during the 12 months of this study.

Discussion

The cryoprecipitation method has several advantages over other methods of preparation of antihaemophilic globulin concentrates: it is simple and not too expensive, requiring neither costly apparatus nor special technical skills; it can be carried out in a closed system, without risk of contamination or need

for sterilization procedures with consequent loss of activity; it results in no wastage of red cells, and can easily be introduced into the routine procedure of hospital blood banks or regional transfusion centres. Moreover, the end-product contains a higher concentration of factor VIII, relative to its total protein content, than most other human antihaemophilic globulin preparations, and bears comparison in this respect with those derived from animal sources, which have the major disadvantage of antigenicity. Because it is most conveniently prepared from individual donor units of plasma, it is particularly appropriate for the treatment of children, for whom one or two packs are often sufficient as a single dose.

During the period of this study we have used cryoprecipitate antihaemophilic globulin for the treatment of bleeding episodes of all degrees of severity, and have experienced no difficulty in attaining factor VIII concentrations up to 100% or more in the patient's plasma when necessary, and maintaining levels above 30%, without circulatory overloading or other adverse effects. The ease of administration of the material in a small volume, by simple intravenous injection, also makes it particularly appropriate for the treatment of relatively minor bleeds, such as haemarthroses, in children, as it obviates the need for setting up a drip; this advantage is especially useful in the treatment of such episodes on an outpatient basis.

Pool and Shannon (1965) observed no measurable decay of activity of their preparation over a six-month period; we have not stored cryoprecipitate as long as this, but have found no appreciable diminution of factor VIII recovery in concentrates stored for at least 10 weeks. The *in-vivo* survival of factor VIII in our preparations compares well with those reported for plasma and for other types of antihaemophilic globulin concentrate. Only one of our 23 patients developed a weak factor VIII inhibitor while receiving cryoprecipitate, with resulting resistance to therapy, though most have received repeated doses over periods of weeks or months. This complication appears only to have been reported in one other case treated with cryoprecipitate antihaemophilic globulin so far (Hattersley, 1966), and does not therefore seem to be provoked more commonly by cryoprecipitates than by other human antihaemophilic globulin preparations.

Our finding that 43% of the factor VIII in the original fresh plasma could be recovered in the patient's circulation, after injection of material prepared by Pool's original method, agrees well with Pool's own estimate (Pool and Shannon, 1965) of 46% *in-vivo* recovery. This latter figure was based on a mean recovery of 70% of the activity of the frozen plasma from which the concentrate was made, which was itself assumed, from the data of Biggs *et al.* (1965), to have about 65% of the activity of fresh plasma. Using our rapid method of preparation, we were able to improve the mean *in-vivo* factor VIII recovery to 61% of that in the original fresh plasma, and this approaches the overall recovery achievable by the transfusion of whole fresh-frozen plasma immediately after rapid thawing. The greater loss of factor VIII during slow thawing is consistent with the finding of Weaver and Langdell (1966) that much more of the original activity was lost while plasma was slowly frozen over a period of 24 hours than during more rapid freezing—an analogous but reverse situation which also produces a slowly changing mixture of frozen and thawed plasma; factor VIII appears to be particularly unstable in these conditions.

It thus appears that almost as good a yield of factor VIII can be obtained from fresh blood as cryoprecipitate as in the form of fresh-frozen plasma—a product over which the concentrated preparation has numerous advantages, of which the most important have been mentioned above. Since the remainder of the blood can be stored and used for ordinary blood-banking purposes, this method provides an economical way of increasing the supply of concentrated human antihaemophilic globulin, and might well supersede the production of fresh-frozen plasma for haemophiliacs by blood transfusion centres.

Because of the wide range of plasma factor VIII concentrations found in normal donors, and the inaccuracy of assays on concentrated material, the best basis on which to calculate the appropriate dose of concentrate for a given situation is the factor VIII concentration of the original plasma from which the cryoprecipitate was prepared. If this is known the dose can be calculated without waiting for the result of an assay performed on the reconstituted material, applying a correction factor to account for the expected recovery, as described above. In practice, however, it may not always be possible to assay the original plasma, and in such a case the following formula may be used as a rough guide:

$$\frac{\text{No. of packs required} = \text{Factor VIII rise intended (\%)} \times \text{body wt. (kg.)} \times 4}{1,000}$$

This is based on the assumptions that a pack of fresh plasma contains 250 units of antihæmophilic globulin, and that the overall in-vivo recovery is not less than 50%, so that 1 unit of antihæmophilic globulin per kg. raises the patient's factor VIII concentration by not less than 1% (see Clinical Results). When the concentration to be achieved is critical, as in major bleeding episodes or to cover operative procedures, it is advisable to check the potency of the material by assaying the concentrate itself. We have found the use of supernatant plasma as the initial diluent for the cryoprecipitate to give more reproducible results in our assay system than other modifications which we have tested.

Summary

Antihæmophilic globulin concentrate was prepared from 278 packs of fresh plasma by Pool's cryoprecipitation method, and from a further 103 packs by a modification of this method, involving more rapid thawing of the plasma. These concentrates were used for the treatment or prevention of bleeding on 59 occasions in 23 hæmophilic patients, including two major surgical operations.

The mean overall recovery of factor VIII in the patient's circulation, relative to the original plasma from which the concentrate was prepared, was 43% for concentrates prepared by Pool's original method and 61% in the case of those prepared

by the rapid modification. This improvement in recovery appeared to be due to a reduction in loss of factor VIII during the faster thawing procedure.

In concentrates prepared by the rapid method the factor VIII was concentrated over twentyfold, and the fibrinogen over tenfold, relative to the total protein. Concentrates were stable on storage at -30°C . for at least 10 weeks. The half-life of factor VIII in the circulation of hæmophilic recipients of cryoprecipitate antihæmophilic globulin varied from 6 to 14 hours.

Excellent clinical results were achieved in all patients treated, except two who had circulating factor VIII inhibitors; one of these developed during treatment with cryoprecipitate antihæmophilic globulin. No other side-effects or reactions were observed. The advantages of this type of human antihæmophilic globulin preparation are discussed, and a guide to the calculation of dosage is given.

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REFERENCES

- Abildgaard, C. F., Cornet, J. A., Fort, E., and Schulman, I. (1964). *Brit. J. Haemat.*, **10**, 225.
 Adelson, E., Rheingold, J. J., Parker, O., Steiner, M., and Kirby, J. C. (1963). *J. clin. Invest.*, **42**, 1040.
 Biggs, R., and Denson, K. W. E. (1963). *Brit. J. Haemat.*, **9**, 532.
 ———, Matthews, J. M., Rush, B. M., Johnstone, F. C., Macfarlane, R. G., and Hayton-Williams, D. S. (1965). *Lancet*, **1**, 969.
 Hardisty, R. M., and Macpherson, J. C. (1962). *Thrombos. Diathes. hæmorrh. (Stuttg.)*, **7**, 215.
 Hattersley, P. G. (1966). *J. Amer. med. Ass.*, **198**, 243.
 Ingram, G. I. C. (1952). *Biochem. J.*, **51**, 583.
 King, E. J., and Wootton, I. D. P. (1956). *Microanalysis in Medical Biochemistry*, 3rd ed., p. 57. London.
 Mollison, P. L. (1962). *Blood Transfusion in Clinical Medicine*, 3rd ed., p. 73. Oxford.
 Pool, J. G., Hershgold, E. J., and Pappenhagen, A. R. (1964). *Nature (Lond.)*, **203**, 312.
 ——— and Robinson, J. (1959). *Brit. J. Haemat.*, **5**, 24.
 ——— and Shannon, A. E. (1965). *New Engl. J. Med.*, **273**, 1443.
 Shulman, N. R., Marder, V. J., and Hiller, M. C. (1962). *J. clin. Invest.*, **41**, 1401.
 Weaver, R. A., and Langdell, R. D. (1966). *Transfusion (Philad.)*, **6**, 224.

Normal Erythrocyte Sedimentation Rate and Age

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It has become more and more important to establish reliable normal values for the biological variables used in clinical medicine. The widespread use of, and demand for, health surveys of population groups has made it necessary to have well-defined limits for "normality" applicable to mass examinations. The increasing number of old people in the hands of the medical profession all over the world also make it necessary to have age-grouped normal values. Values now commonly in use have been employed to distinguish between health and well-established disease in individual patients and are generally not correlated to age.

At least in the Scandinavian countries, the erythrocyte sedimentation rate (E.S.R.) performed by the Westergren method has been one of the most commonly employed tests to separate "disease" from "health." Westergren's original

normal values (men 3 mm., women 7 mm./hr.) are now generally thought to be too low to be of practical value, especially in older people. Many have had the impression that there is a general increase of the E.S.R. with age, but it is always difficult, sometimes impossible, to state whether such an increase is due to hidden disease or to "normal" age factors. A large group of healthy people—in active work—regularly examined at a health survey centre offered an excellent opportunity to study the influence of age on the E.S.R.

Material and Methods

The material was obtained from a health survey centre (Företagens Hälsokontroll, Stockholm). This centre makes regular examinations, at yearly intervals, of employees of various companies that have a contract with the centre. Those who come for their routine visits are all engaged in active work

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