Treatment of haemophilia A with purified factor VIII obtained from human plasma by cryoprecipitation

Treatment of Haemophilia A with purified Factor VIII obtained from Human Plasma by Cryoprecipitation

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A few years ago it was discovered by coincidence that so-called cryoprecipitate – a protein fraction which precipitates from human plasma at a low temperature under certain conditions – contains an abundance of a clotting factor which is lacking in haemophilia A, and which is known by international agreement as Factor VIII[12]. In view of an exceedingly encouraging report from the USA[3] on the clinical validity of cryoprecipitate in the treatment of haemophilia A, we started the production of this fraction in December 1965. Our purpose was to establish whether cryoprecipitate would be suitable for the treatment of haemophiliacs, particularly in the case of an operation or after severe injuries, in which instances the conventional products (fraction I according to COHN, fraction I-O according to BLOMBÄCK, animal fractions) had been only partly satisfactory[10].

In order to evaluate cryoprecipitate, we determined both the yield and degree of purity *in vitro*, and the activity of purified Factor VIII *in vivo*, with special reference to the relation between the quantity administered and the resulting blood concentrations.

Finally we attempted to establish whether transfusion of cryoprecipitate exerts any influence on the degree of physiological Factor VIII production in patients with the mild type of haemophilia A (with a Factor VIII level of 5-25 pct).

METHODS

Procedure of cryoprecipitate production[12, 15]

Some 500 ml blood is collected under vacuum into one of the two bags of a double-bag plastic system*. Such a system consists of a main bag and a satellite bag, interconnected by plastic tubing. The blood is collected into the main bag, the collecting time being generally less than 6 minutes. The bags are centrifuged for 30 minutes at 4°C at 4000 × *g*. A plasma extractor is then used to transfer the plasma (current routine production is about 280 grams per bag) to the satellite bag. The latter is subsequently suspended for 20-30 minutes in a CO_2-alcohol mixture at −60° to −30°C. The two bags, still connected, are then placed in a refrigerator at 4°C. After 20-24 hours the slowly thawing plasma contains a floccular precipitate: the so-called cryoprecipitate.

The bags are then centrifuged for 20 minutes at 4°C at 3000 × *g*, in the course of which the precipitate attaches itself to the bottom and lateral walls of the satellite bag. The plasma is allowed to flow back into the erythrocyte concentrate in the main bag, while the cryoprecipitate remains in the satellite bag. The bags are then clamped off and separated. Satellite bags with the cryoprecipitate are stored at low temperature, originally at −25°C, currently at −70°C.

To prepare the cryoprecipitate for transfusion purposes, the required number of bags are taken from the deep freezer and placed for one minute in a water bath at 37°C to soften the plastic. The bags are opened by severing the (iodinized) tubing with sterile scissors, whereupon a syringe is used to add about 5 ml of a sterile buffer solution (1 part 0.109 M sodium citrate and 5 parts 0.9 pct NaCl solution) to each bag. To ensure rapid solution of the cryoprecipitate, the tubing is clamped off with a Kocher clamp and the bags are placed for a few minutes in a water bath at 37°C. The contents are then poured into a sterile siliconized bottle and the bags are rinsed out at least twice with the same amount of citrated salt solution. The ultimate volumes of 10 bags total 150-200 ml, a few ml of which is used for bacteriological examination and determination of Factor VIII activity.

*Initially we used Fenwal double blood-pack units J-D2, but later on those supplied by the Blood Transfusion Service of the Netherlands Red Cross, Amsterdam which contain 75 ml of anhydrous di-sodium citrate 2.7 pct and anhydrous dextrose 2.3 pct.

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Factor VIII assay

Equipment: Coagulometer for automatic recording of coagulation times according to SCHNITGER[17] (Depex, de Bilt, Netherlands); facilities for centrifugation at 4°C and 20.000 × *g*; glassware and plastics: siliconized blood bank bottles (500 and 350 ml); parafilm "M" (Div. of Am.Can.Comp., Wisconsin, USA); calibrated pipettes; ordinary glass, disposable, round bottom tubes (9.5-11.7 × 55 mm) for use in the coagulometer (Depex, de Bilt, Netherlands); ordinary glass, siliconized, round bottom tubes (16-17.5 × 100 mm) used for preparing dilutions; polystyrol crystal, flat bottom tubes (15-17 × 56 mm) with polythene cap, for collecting blood and storing plasma samples (Emnosa, Zuun, Belgium).

Reagents: Veronal buffer pH 7.4, 0.05 M, according to MICHAELIS; Calcium chloride 0.033 M; Light kaolin (British Drug Houses, Poole, Great Britain); Phospholipid according to BELL and ALTON[1], stored in small amounts at −25°C; Sodium citrate 0.55 M (about 20 pct solution).

Normal pooled plasma: From each of 4 normal individuals known to display a mean Factor VIII activity of about 100 pct, 100 ml blood was collected on 2 ml sodium citrate 0.55 M, and thoroughly mixed before centrifugation for 30 minutes at 1250 × *g*. The supernatant plasma was pooled, centrifugated at 20.000 × *g* for 30 minutes at 4°C and stored in 1 ml portions at −70°C. This procedure takes about two hours.

Test plasma: Blood was collected in a polystyrol tube containing 0.55 M sodium citrate (0.1 ml for 4.9 ml blood). After thorough mixing the blood was centrifugated at 1250 × *g* for 15 minutes. Platelets were almost completely removed from the supernatant plasma by centrifugation for 30 minutes at 4°C at 20.000 × *g*.

Reagent plasma: From a patient with a severe type of haemophilia A (< 1 pct Factor VIII), blood was collected in a siliconized blood bank bottle (60 ml ACD for 440 ml blood), gently mixed during collection and centrifuged for one hour at 4°C at 1800 rpm. The supernatant plasma, practically freed from platelets by centrifugation for 30 minutes at 20.000 × *g*, was stored in small amounts at −25°C.

Procedure: A modification of the one-stage method as described by HARDISTY and MCPHERSON[4] was adapted for use in the coagulometer: a series of test tubes are filled successively with 0.1 ml of freshly thawn reagent plasma, 0.1 ml of a dilution of normal or test plasma, and 0.1 ml of a kaolin suspension (5 mg/ml phospholipid solution). The contents are thoroughly stirred and the tubes are then placed in the thermostat housing of the coagulometer. Maximum contact activation is achieved after an incubation period of 30 minutes[20]. The reaction is then started by admixing 0.1 ml pre-heated $CaCl_2$ 0.033 M, and the coagulation times are recorded by the coagulometer. All determinations are made in triplicate, the second and third series (Fig. 1) being prepared during the incubation period of the first. The Factor VIII activity in the test plasma is then read from the standard curve obtained by plotting the logarithm of the calculated Factor VIII content in dilutions of normal plasma against the logarithm of the clotting times observed with these dilutions. Dilutions of the normal reference plasma are always chosen so as to give a range of clotting times that includes the clotting times found with the test sample, in order to avoid extrapolation of the standard curve. Statistical analysis of over 100 determinations [5, 20] failed to demonstrate any difference between the standard curve in the range used, and a straight line. The experimental error of this method is between 5 pct and 8 pct.

Fibrinogen determination

A quantitative immuno-precipitation technique was used[9, 16].

Protein determination

According to KJELDAHL.

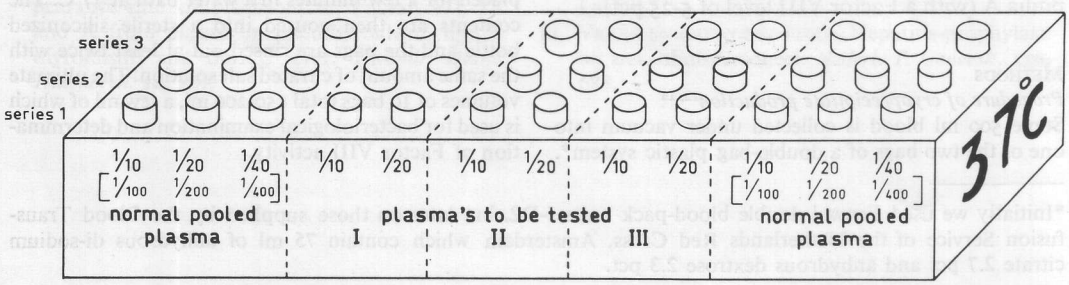

Fig. 1. Principle of Factor VIII-assay with the use of SCHNITGER's coagulometer. The three series serve for triplicate determination of the samples to be tested (for further explanation see text).

TABLE I: CLINICAL DATA OF 9 HAEMOPHILIA A PATIENTS TRANSFUSED WITH CONCENTRATED FACTOR VIII

Patients	*Age (years)*		*Factor VIII level (pct)*	*Reason for admission*	*Blood vol. (l)*	*Haematocrit (pct)*	*Plasma vol. (l)*	*Body temp.** (C°)*
P. J. J.	52	I*	< 1	muscular		36	2.56	37.7
		II		haematoma	3.78	40	2.42	37.6
P. K.	36	I	< 1	haematuria	5.23	38	3.44	37.0
		II				39	3.39	37.5
G. v. W	32	I	< 1	tooth	4.01	46	2.35	37.5
		II		extraction		46	2.35	37.2
J. C. O.	42	I	< 1	haemarthros	5.07	44	3.06	37.1
		II				42	3.15	37.5
H. P. R.	35	I	< 1	haemarthros	4.88	35	3.34	37.1
		II				38	3.21	37.3
H. v. M.	18	I	< 1	haematuria	4.01	33	2.82	37.4
		II				33	2.82	37.2
A. P.	46	I	6.0	haematuria	5.65	34	3.92	37.1
		II	8.9			33	3.97	37.0
		III	14.8			21	4.58	37.2
		IV	11.1			33	3.97	37.1
		V	14.0			31	4.07	37.3
		VI	10.0			30	4.12	37.0
J. L.	20	I	6.0	tooth	6.05	43	3.71	38.2
		II	12.5	extraction		45	3.60	37.5
		III	6.4			44	3.65	37.2
		IV	6.8			45	3.60	37.4
B. J. B.	17	I	3.4	tooth	5.01	42	3.12	37.9
		II	3.5	extraction		42	3.12	38.1

*Roman numerals refer te the number of transfusion experiments.
**Maximum rectal temperature registered during the day of the transfusion; all the other data relate to the situation prior to transfusion.

PATIENTS

Cryoprecipitate was tried out in 10 patients suffering from haemophilia A. Nine of them were adult volunteers; the tenth was a 6-year-old boy who, after consultation with the parents, was subjected to a herniotomy under cryoprecipitate protection.

Six of the 9 adults suffered from a severe type of haemophilia A (< 1 pct Factor VIII), while the other 3 showed the mild type of haemophilia A (Factor VIII 5-25 pct). An indication for treatment existed in all cases. Five of the 6 patients with a severe type of haemophilia A were clearly "polytransfusees", whose risk of transfusion hepatitis could be regarded as minimal.

Table I presents data on the nine adult haemophiliacs, including data on the blood volume as determined with the aid of Cr^{51}-labelled erythrocytes (the plasma volume in these patients was calculated from blood volume and haematocrit value; the body's haematocrit value was estimated 10 pct lower than the value obtained for peripheral blood).

The surgical patient F. v. L. was the second son of a known carrier of mild type haemophilia. In his case Factor VIII activity before the operation was about 10 pct of normal; his body weight was 22 kg, and on the basis of this weight the plasma volume was estimated at 0.88 l. The operative indication was a right-sided inguinal hernia which had become incarcerated several times.

RESULTS

Properties of cryoprecipitate in vitro

Factor VIII yield; Concentration of fibrinogen and other proteins: From the yield of 28 samples, each derived from 10 pooled cryoprecipitates, it was calculated that the yield per cryoprecipitate averages 44.8 pct of the Factor VIII activity, 41.1 pct of the fibrinogen concentration and 1.4 pct of the concentration of other proteins present in the original plasma. Assuming that the amount of

TABLE II: RECOVERY IN VIVO OF FACTOR VIII TRANSFUSED AS A CRYOPRECIPITATE

	Mean plasma volume (l)	*Number of transfusions*	*Mean amount and range of Factor VIII* transfused*	*recovered*	*Average recovery (pct)*
Severe-type haemophilia (6 patients)	2.91	12	901 (504-1620)	929 (623-1186)	103
Mild-type haemophilia (3 patients)	3.79	12	917 (547-1254)	854 (659-1154)	93

*Expressed as equivalents of freshly drawn net normal plasma in ml.

original plasma per cryoprecipitate averages 244 ml ACD plasma (=250 g), i.e. 194 ml net plasma, this means that – in terms of Factor VIII, fibrinogen and other protein concentrations – one cryoprecipitate corresponds with 87, 80 and 2.6 ml fresh plasma, respectively. The average total protein concentration per cryoprecipitate is approximately that of 7 ml fresh net plasma. The coefficient of variation of the Factor VIII activity found per 10 pooled bags was calculated to be 16 pct. Given an error of determination of 7 pct, this means that the coefficient of variation for the yield per 10 pooled bags amounts to some 14 pct ($\sqrt{256-49}$ pct) and for the yield per bag to about 45 pct (14 pct $\times$ $\sqrt{10}$). The Factor VIII yield per donor, therefore, varies widely.

Iso-antibody concentration: In two random samples of 10 pooled cryoprecipitates (200 and 180 ml, respectively), obtained from donors with blood group O, determinations disclosed titres of $^1/_{16}$ and $^1/_{16}$, respectively, for complete antibody anti-A, and $^1/_8$ and $^1/_4$, respectively, for anti-B; incomplete antibodies and haemolysins were not demonstrable.

Activity of purified Factor VIII in vivo

Recovery: Each of the six adult patients with severe haemophilia A received two infusions of 10 pooled cryoprecipitates, usually with an interval of only a few days. The three patients with mild haemophilia A likewise received repeated infusions of 10 pooled cryoprecipitates (the first being given six, the second receiving four and the third receiving two infusions). The duration of the infusion was 15-20 minutes in all cases.

Ten minutes after completion of the infusion a separate venipuncture was performed to collect blood for determination of the Factor VIII concentration attained. The recovery was calculated by relating the value found *in vivo* to the value, predicted on the assumption of a homogeneous distribution over the intravascular plasma volume of the amount of cryoprecipitate administered. The latter was obtained from determinations *in vitro* (Table II). Statistical analysis showed that both in the group with severe and in that with mild haemophilia, the average recovery is not significantly different from 100 pct.

Distribution and degradation: In order to determine the rate of clearance of the transfused Factor VIII from the blood, the Factor VIII activity was measured at regular intervals during the first 32 hours after administration. Table III presents a summary of the individual values found. In none of the patients did an exceptionally rapid clearance of Factor VIII suggest the presence of an iso-immune antibody against Factor VIII. Figures 2 and 3 show Factor VIII clearance curves for the group with severe and that with mild haemophilia A, respectively. The curves are the optimal lines drawn through the geometric means calculated for the two groups of patients from the values indicated in Table III as found at corresponding times. In calculating the points for Figure 3 after infusions I and III in patient J. L., only the first two values were used

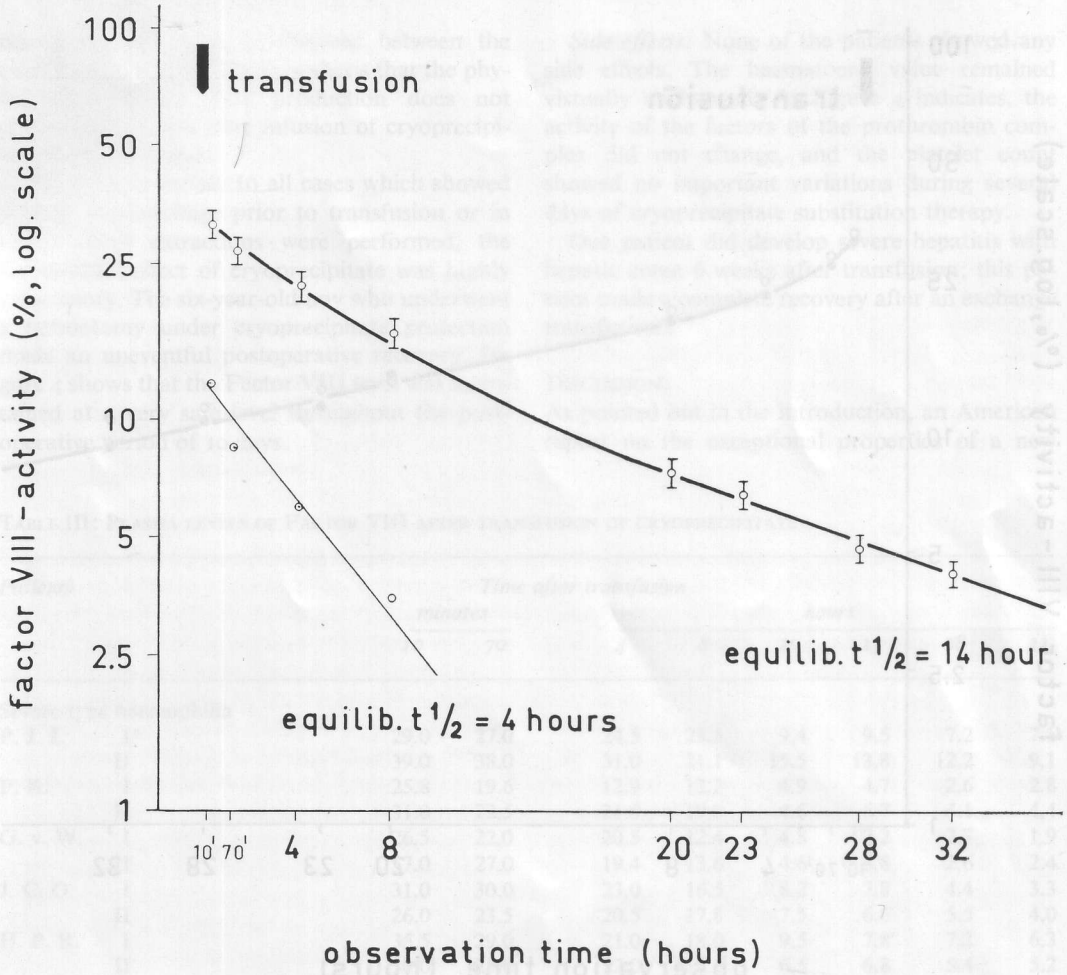

Fig. 2. Disappearance rate of concentrated Factor VIII contained in cryoprecipitate after transfusion into patients suffering from severe haemophilia. Each point with deviation $\dot{\pm}$ represents the geometric mean of the results obtained from 12 observations in 6 patients and the averaged 95 pct confidence interval calculated for the 8 geometric means.

because the second follow-up was followed by a tooth extraction under local anaesthesia with procaine and adrenaline (about 0.04-0.08 mg), whereupon Factor VIII activity in both cases showed a significant increase.

The clearance curves presented in Figures 2 and 3 are both diphasic: Factor VIII activity showed a higher rate of diminution during the first 24 hours than subsequently. On statistical grounds the data obtained in patients with severe haemophilia A warrant the following assumptions: 1) the rate of clearance during the second phase takes an exponential course with a biological half-life of 14 hours, and 2) the first phase is characterized by summation of the slow inactivation mentioned under 1) and a second, more rapid, possibly also exponential clearance (probably the result of equilibration between the intravascular and the extravascular distribution space), the half-life of which is estimated at 4 hours (see Fig. 2).

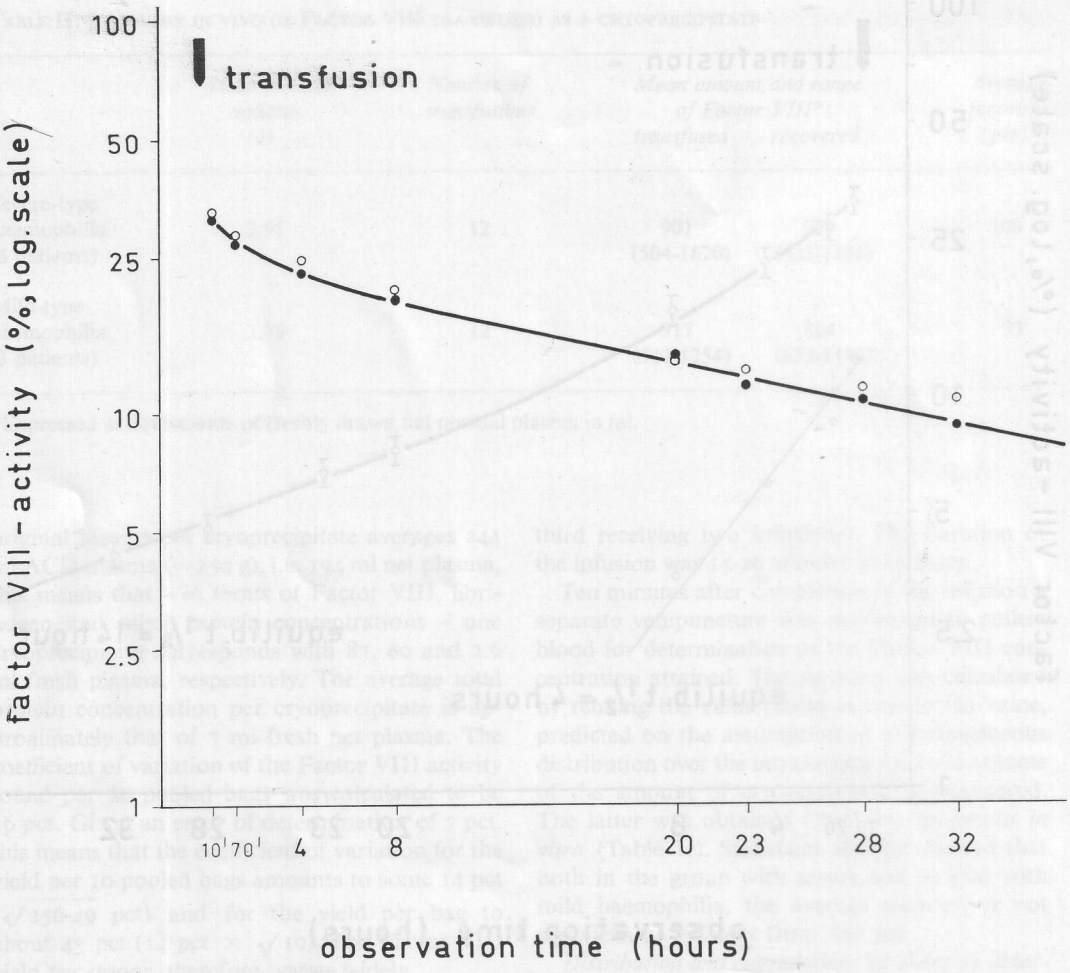

Fig. 3. Disappearance rate of concentrated Factor VIII transfused into patients suffering from mild haemophilia Each point represents the geometric mean of the results obtained from 12 observations in 3 patients.

The curve in Figure 3 differs from that in Figure 2 in two respects. Firstly, the maximum rise (10-minute value reduced by the mean pre-value of 7.8 pct) with 24 pct is obviously lower than that found for the group of severe haemophiliacs. Secondly, the slope of the second phase of the clearance curve is definitely less steep. The former difference is explained by the considerably larger intravascular plasma volume in the three mild haemophiliacs. The flatter clearance curve might be explained by assuming that the physiological Factor VIII production remains unchanged during and after infusion. To verify the latter hypothesis, the values found for severe haemophiliacs (Fig. 2) were converted on the basis of the mean plasma volume in the mild haemophiliacs (the plasma volume in the severe haemophiliacs was two-thirds of that found in the mild haemophiliacs), and increased by the mean pre-value of 7.8 pct Factor VIII activity. The results of this calculation are indicated by the circles in Figure 3. At corresponding times, no statistically

convincing difference is observed between the circles and the dots. Our hypothesis that the physiological Factor VIII production does not change during and after infusion of cryoprecipitate therefore stands.

Haemostatic action: In all cases which showed definite haemorrhage prior to transfusion or in which tooth extractions were performed, the haemostatic effect of cryoprecipitate was highly satisfactory. The six-year-old boy who underwent a herniotomy under cryoprecipitate protection made an uneventful postoperative recovery. Figure 4 shows that the Factor VIII level was maintained at a very safe level throughout the postoperative period of 10 days.

Side effects: None of the patients showed any side effects. The haematocrit value remained virtually unchanged. As Figure 4 indicates, the activity of the factors of the prothrombin complex did not change, and the platelet count showed no important variations during several days of cryoprecipitate substitution therapy.

One patient did develop severe hepatitis with hepatic coma 6 weeks after transfusion; this patient made a complete recovery after an exchange transfusion[2].

Discussion

As pointed out in the introduction, an American report on the exceptional properties of a new

Table III: Plasma levels of Factor VIII after transfusion of cryoprecipitate

Patients		*Time after transfusion*							
		minutes		*hours*					
		10	*70*	*4*	*8*	*20*	*23*	*28*	*32*
Severe-type haemophilia									
P. J. J.	I*	29.0	27.0	24.5	23.5	9.4	9.5	7.2	7.1
	II	39.0	38.0	31.0	21.1	15.5	13.8	12.2	9.1
P. K.	I	25.8	19.6	12.9	12.2	4.9	4.7	2.6	2.8
	II	31.0	22.5	21.5	19.0	4.6	6.7	4.1	4.4
G. v. W.	I	26.5	22.0	20.5	12.4	4.8	2.2	2.7	1.9
	II	37.0	27.0	19.4	13.6	4.6	3.8	2.6	2.4
J. C. O.	I	31.0	30.0	23.0	16.5	8.2	7.8	4.4	3.3
	II	26.0	23.5	20.5	17.8	7.5	6.6	5.5	4.0
H. P. R.	I	35.5	29.0	21.0	18.0	9.5	7.8	7.2	6.3
	II	30.0	25.0	22.0	18.5	6.5	6.8	5.4	5.2
H. v. M.	I	38.0	33.0	29.0	15.7	8.7	8.0	4.7	4.1
	II	37.0	33.0	26.0	17.1	9.5	7.9	4.1	3.5
Mild-type haemophilia									
A. P.	I	28.0	23.0	18.7	16.7	13.7	11.1	12.6	11.5
	II	25.5	24.0	22.5	21.5	17.0	16.7	11.8	10.0
	III	33.5	30.5	27.0	24.0	23.0	19.4	18.0	18.0
	IV	28.0	23.5	23.5	20.0	21.0	16.3	15.6	11.8
	V	31.0	22.0	23.5	24.0	20.0	16.6	15.3	12.5
	VI	38.0	32.5	26.5	25.0	20.3	16.5	13.6	11.0
J. L.	I	28.5	25.0	**43.0**	**26.7**	**23.0**	**13.0**	**8.7**	**10.3**
	II	31.0	32.0	20.0	18.6	10.4	10.7	9.9	9.3
	III	35.8	31.5	**37.5**	**20.3**	**11.3**	**10.0**	**10.0**	**7.1**
	IV	26.0	22.0	21.0	14.5	9.8	7.6	7.9	6.8
B. J. B.	I	34.0	31.0	26.0	18.0	7.5	7.4	6.0	4.6
	II	39.8	32.0	21.5	16.0	8.0	5.2	4.8	4.0

*Roman numerals refer to the number of transfusion experiments.
For pretransfusion levels see Table I and for explanation of the figures in heavy print see text.

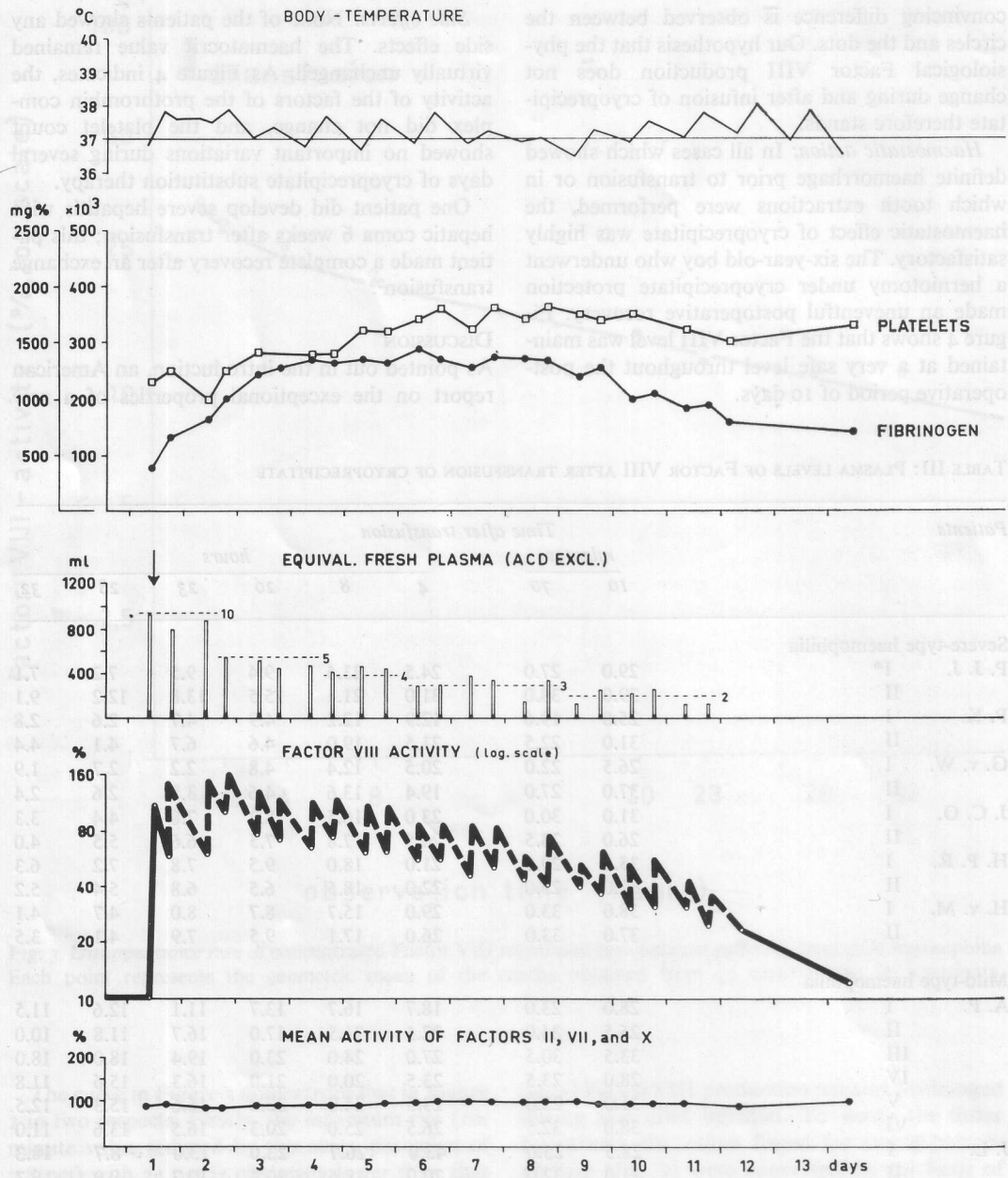

Fig. 4. Data concerning treatment and clinical course of a patient suffering from haemophilia A operated upon for an inguinal hernia. Arrow indicates operation.

human Factor VIII product called cryoprecipitate[6,12,13,14,15] led to the above described preliminary study. The results warrant several conclusions, primarily with regard to the properties of cryoprecipitate.

The Factor VIII activity in cryoprecipitate, as

determined *in vitro*, amounts to almost 45 pct of that in the original plasma. Ten pooled cryoprecipitates average a value equivalent to that of well over 900 ml fresh net plasma, with a standard deviation of about 150 ml.

The Factor VIII: fibrinogen ratio in cryoprecipitate is the same as that in fresh plasma but the concentration of other proteins is low so that, on a protein basis, Factor VIII is about 13 times more concentrated in comparison with fresh plasma. With the procedure we used, the concentration on a volume basis is about 6 times, but can easily be increased to 10 times. This means that neither the protein concentration nor the volume of cryoprecipitate is incompatible with optimal substitution therapy. This cannot be maintained quite so categorically with regard to fraction I according to COHN (two-donor fibrinogen) or fraction I-O according to BLOMBÄCK[10], in which the Factor VIII: fibrinogen ratio is often considerably smaller than that in cryoprecipitate.

The Factor VIII contained in cryoprecipitate showed a normal behaviour *in vivo:* recovery is virtually complete (Table II), the rate of clearance from the blood after transfusion in patients with severe haemophilia A is normal (half-life in the slow phase is 14 hours; Fig. 2), and the haemostatic effect is excellent. The haemostatic effect was satisfactory not only in the patients described in this paper but also in three other cases that we observed recently. One was a case of severe haemophilia in which 70 cryoprecipitates were given within a week in the course of the treatment of a haematoma of the intestinal wall with ileus. The second was a little boy suffering from mild haemophilia A who received 65 cryoprecipitates and the third was a case of female haemophilia in which a total of 91 cryoprecipitates were given in an attempt to ensure post-tonsillectomy wound healing.

The stability of Factor VIII in cryoprecipitate at —25°C appears to be satisfactory. There are no indications of diminution of the activity after a few month's storage at this temperature. But further pertinent investigation is required.

No side effects have been observed in the course of and after transfusions which have so far totalled over 650 cryoprecipitates in 13 different patients. No haemolytic transfusion reaction need be expected because blood group compatibility is taken into account. However, even for patients of blood groups B and AB who receive cryoprecipitate from blood group O donors, the risk of haemolytic reactions is exceedingly small because the concentrations of incomplete antibodies and haemolysins are so low as to be beyond the reach of conventional measuring techniques.

The one instance of hepatitis occurred in a man with severe haemophilia A who had hardly received any transfusions in the past and had not been given γ-globulin prophylactically. In terms of frequency of transfusions, this patient could be compared with a mild haemophiliac, who receives only sporadic transfusions and as a rule has not (yet) had transfusion hepatitis. In the event of intensive substitution therapy it is therefore advisable to resort to γ-globulin prophylaxis for this group[11,21], the first dose to be given intramuscularly during substitution, and the second dose subcutaneously 4 weeks after the last infusion. A reduction of the hepatitis risk can be ensured in a different (and perhaps more adequate) way by producing cryoprecipitate from a small number of donors with the aid of plasmapheresis[21].

Cryoprecipitate is inexpensive because the production of this Factor VIII preparation is considerably less complicated than any other procedure of purification so far used (a laboratory technician who has the disposal of a single centrifuge for centrifuging the double bags can produce as many as 40-60 cryoprecipitates per week without difficulty, including the collection of blood and the preparation of preserved blood). Furthermore, after isolation of the cryoprecipitate, the remainder of the blood retains its usefulness for nearly all indications (over 50 pct fibrinogen remains in the plasma, and the Factor VIII concentration of the preserved blood is rarely of importance for the recipient).

Partly on the basis of previous studies[7,8] the following can be stated about *the amount of cryoprecipitate required for adequate substitution:* After administering a booster dose at the start of transfusion treatment and subsequent continuous infusion of cryoprecipitate, it will take about two days before the Factor VIII distribution in the intravascular space is in balance with that in the extravascular space. The amount of Factor VIII administered during these two days can be called the charging dose, while the subsequent dose may be described as maintenance dose. The following

hypothetical case may serve as an example. In an adult patient with severe haemophilia A whose intravascular plasma volume amounts to 2.5 l, the charging dose will be started by rapid administration (20-30 minutes) of 15 pooled cryoprecipitates, which ensures a blood concentration of 40-70 pct (15 cryoprecipitates contain an amount of Factor VIII equivalent to 1000-1700 ml net fresh plasma). During the first two days after the starting dose, a constant drip infusion of the equivalent of 1750-2750 ml net fresh plasma daily (i.e. 25 cryoprecipitates daily) will maintain the Factor VIII concentration in the plasma at 40-60 pct. The maintenance dosage can be started on the third day of substitution therapy once a constant blood level is ensured because the amount administered balances degradation. With a constant drip infusion, the daily amount H of Factor VIII to be transfused – in equivalents of ml freshly drawn normal net plasma – can be approximated as follows[7]:

$H = 17 \times$ intravascular plasma space (in liters) $\times$ desired blood level (pct)

The constant 17 is derived from

$$\frac{0.693 \times 24 \times 1.4 \times 1000}{14 \times 100}$$

in which 0.693 represents the natural log. of 2, 24 the duration of infusion in hours, 1.4 the correction for the initial rapid disappearance of Factor VIII (half-life 4 hours), and 14 the biological half-life of Factor VIII in hours.

In the haemophiliac with a plasma volume of 2.5 l presented as an example, therefore, maintenance of a 40 pct concentration during the 3rd and 4th day of substitution requires the equivalent of some 1700 ml net fresh plasma (averaging almost 20 cryoprecipitates daily). During the subsequent 10 days, the equivalent of 1275 ml (averaging almost 15 cryoprecipitates daily) will suffice to maintain an average concentration of 30 pct, with minimum values not below 25 pct. The total number of cryoprecipitates required for substitution will in this case be about 250; in terms of Factor VIII concentrations in plasma this guarantees a safe margin especially during the first four days of substitution. In actual practice it may of course be necessary to modify the dosage, dependent on the results of Factor VIII assays in the patient's blood. These assays should be carried out at least twice a day during substitution therapy. Adjustment of the dosage is most likely to be necessary in the event of an increased catabolism as occurs in complicated wound healing or in the presence of other disturbances in the postoperative or posttraumatic course. In these cases the Factor VIII requirement may well increase to twice the normal dosage[8] because the Factor VIII consumption is twice as high. Before starting planned substitution therapy, therefore, it must be ensured that the number of cryoprecipitates available amounts to twice the number calculated for a normal catabolism; in the above theoretical example this number of cryoprecipitates would be about 500.

From the considerations given above a *general dosage scheme* can be derived for an adult patient, assuming a mean yield of 45 pct Factor VIII per cryoprecipitate, and no *in vitro* deterioration of Factor VIII during continuous drip infusion (Table IV). In the case of children the dosage scheme is essentially the same but owing to the smaller number of cryoprecipitates administered daily the large variation in Factor VIII yield per cryoprecipitate already mentioned will lead to less predictable plasma levels for Factor VIII.

Nevertheless, the values found in the case of the boy with mild haemophilia A who underwent a herniotomy, show that our estimate of the number of cryoprecipitates required for adequate substitution therapy is not far from the actual amount administered. In this boy with an esti-

TABLE IV: GENERAL DOSAGE SCHEME FOR ADMINISTRATION OF CRYOPRECIPITATE

	cryoprecipitates per kg body weight
Initial dose* (rapid infusion)	0.24
First 2 days	0.40-0.80 daily
3rd and 4th day	0.30-0.60 daily
Subsequent 10 days	0.23-0.46 daily

*Of course a test dose will be given a few hours (or a day) before a planned surgical intervention in order to rule out a circulating anti-Factor VIII anticoagulant. For this purpose the adult patient may be given 10 pooled cryoprecipitates; the blood Factor VIII concentration is then determined 10 minutes and 70 minutes after this administration.

mated plasma volume of 0.88 l, daily administration of an amount of Factor VIII which averaged the equivalent of 800 ml net fresh plasma, ensured an average Factor VIII concentration roughly estimated as 60 pct (Fig. 4). As calculated with the aid of the above equation, the theoretical prediction (assuming continuous infusion, the same Factor VIII turnover as in adults and an unchanged physiological Factor VIII production of 10 pct) would have been:

$$10 \text{ pct} + \frac{800}{0.88 \times 17} \text{ pct} = 63.5 \text{ pct}$$

Many further observations on substitution with continuous drip infusion will of course be required to verify the correctness of the dosage scheme given above.

The fibrinogen concentration to be expected during substitution is dependent on the physiological fibrinogen production and the amount of fibrinogen transfused. In the event of a complicated postoperative or posttraumatic course, the physiological production can of itself result in a blood concentration exceeding 1000 mg/100 ml. To this, the transfused fibrinogen adds about 1000 mg/100 ml, assuming that the distribution space for fibrinogen is the same as that for Factor VIII, that the biological half-life of fibrinogen is 100 hours and that, after the first 4 days, the amount of cryoprecipitate given does not exceed that required to maintain a Factor VIII concentration of 30 pct. The correctness of these assumptions is supported by the case of the boy, in whom a maximum fibrinogen concentration of 1500 mg/100 ml was observed during an uneventful postoperative course (Fig. 4). Since a blood fibrinogen concentration of 2000-3000 mg/100 ml has no untoward effects, fibrinogen will not be a limiting factor in substitution therapy with cryoprecipitate.

Finally it may be pointed out that, especially with a view to reducing the hepatitis risk, attempt must be made (apart from a plasmapheresis programme) to raise the Factor VIII yield per donor. Moreover, a study should be made of the stability of cryoprecipitates, both in deep-frozen dissolved form and when lyophilized. It may be possible also to use cryoprecipitate as the starting material for the preparation of a highly concentrated human Factor VIII product.

SUMMARY AND CONCLUSIONS

An evaluation was made of the Factor VIII activity of some 650 cryoprecipitates.

In vitro, pools of 10 cryoprecipitates have a Factor VIII activity which equals that of 900 (± 130) ml freshly drawn normal plasma. The average yield of Factor VIII is nearly 45 pct; the Factor VIII: fibrinogen ratio remains unchanged. Considered on a protein basis, Factor VIII in cryoprecipitate can be described as about 13-fold purified.

In vivo, the recovery of Factor VIII following a single transfusion of 10 cryoprecipitates does not differ from 100 pct, regardless of the severity of the transfusee's haemophilia. During the first 24 hours after transfusion, Factor VIII disappears from the blood much more rapidly than during the next 24 hours. The initial rapid disappearance can be regarded as due to a combination of equilibration between the intravascular and the extravascular space (half-life 4 hours) and biological decay. The slower degradation during the second 24 hours may be regarded as due merely to biological decay (half-life 14 hours). The haemostatic effect, observed in a case of herniotomy and in one of tonsillectomy, was excellent.

Our observations indicate that a minimum requirement of 250 cryoprecipitates will be needed if major surgery is to be covered safely in an adult with severe haemophilia. The requirement in mild haemophilia is less because the rate of (defective) Factor VIII production does not seem to be altered by cryoprecipitate infusions.

Apart from one case of hepatitis, no untoward reactions were observed. Neither fibrinogen nor iso-antibodies appear to be limiting factors for this transfusion therapy.

First-rate quality, outstanding simplicity of preparation and blood bank economy are the reasons why cryoprecipitates are now being produced on a routine scale in our hospital.

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ADDENDUM: After the preparation of this manuscript two publications have appeared which further substantiate the great value of cryoprecipitate preparations:

BARRETT, K. E., M. C. G. ISRAËLS and A. M. BURN (1967) The Effect of Cryoprecipitate Concentrate in Patients with Classical Haemophilia *Lancet I*, 191.

PRENTICE, C. R. M., R. T. BRECKENRIDGE, W. B. FORMAN and O. D. RATNOFF (1967) Treatment of Haemophilia (Factor VIII deficiency) with Human Antihaemophilic Factor Prepared by the Cryoprecipitate Process. *Lancet I*, 457.

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