SEROLOGICAL PRODUCTS LIMITED

6th FLOOR
REGINA HOUSE
5 QUEEN STREET
LONDON EC4N 1SP

Telephone 236 0488/9

Mrs. Diernhofer,
Immuno AG.
Industriestrasse 72
A-1220 Vienna,
Austria.

NB/JB 11th. December, 1972

Dear Mrs. Diernhofer,

We enclose a replacement set for you to exchange with those at present in your copy of the Kryobulin submission.

Yours sincerely,
for SEROLOGICAL PRODUCTS LIMITED

p.p. GRO-C

MANAGING DIRECTOR

AN APPLICATION
BY
SEROLOGICAL PRODUCTS LTD.
FOR A PRODUCT LICENCE
FOR

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

CONTENTS

more than 3.0 per cent. w/v of total protein, not more than 200 milliequivalents of sodium ions per litre and not more than 165 milliequivalents of citrate ions per litre, with the exception of KRYOBULIN™ 500 units of Factor VIII to be reconstituted with only 20 ml of Water for Injections, B.P. which contains up to 3 % w/v of fibrinogen and up to 6 % w/v of total protein.

In fact, the individual strengths of KRYOBULIN™ are composed as follows:

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
100 units of Factor VIII
to be reconstituted with 10 ml of Water for Injections B.P.

1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3.0 % w/v
- thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	267.0 m. moles/litre
Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
250 units of Factor VIII
to be reconstituted with 20 ml of Water for Injections B.P.

1 ml of the solution contains 12,5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v
- thereof Fibrinogen	1 - 1.5 % w/v
Glycine	267.0 m. moles/litre
Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 20 ml Water for Injections B.P.

1 ml of the solution contains 25 units (± 10 %) of Factor VIII.

Total protein	4 - 6 % w/v
- thereof Fibrinogen	2 - 3 % w/v
Glycine	267.0 m.moles/litre
Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 40 ml Water for Injections B.P.

1 ml of the solution contains 12.5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v
-thereof Fibrinogen	1 - 1.5 % w/v
Glycine	267.0 m.moles/litre
Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 100 ml Water for Injections B.P.

1 ml of the solution contains 5 units (± 10 %) of Factor VIII.

Total protein	0.8 - 1.2 % w/v
- thereof Fibrinogen	0.4 - 0.6 % w/v
Glycine	267.0 m.moles/litre
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

1000 units of Factor VIII

to be reconstituted with 100 ml Water for Injections B.P.

1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3 % w/v
- thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	267.0 m.moles/litre
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

10. PHYSICAL CHARACTERISTICS

A white to yellowish amorphous powder or friable solid without any characteristic odour.

11. CLINICAL USE

a) Recommended clinical use:

Treatment of bleeding caused by Factor VIII deficiency in patients with:

- Haemophilia A
- von Willebrand's Disease
- Haemophilia caused by Factor VIII inhibitors
- Thrombocytopenia with decreased Factor VIII activity
- Combined coagulation disorders, also including reduced Factor VIII activity (consumption coagulopathy, autoimmune diseases, neoplasms , etc.).

A detailed description of treatment, precautions and treatment of any reaction mentioned above is contained in the circular enclosed in each pack sold.

Registration of KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC FRACTION has been applied for at the Health Authorities of the following countries:

Federal German Republic
Italy
Spain
Portugal
Argentine
Brazil
Peru
U.S.A.

KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC FRACTION has been registered with the Health Authorities of the following countries:

Greece	Lic. No. 6 10053/71
Chile	Lic. No. 1.433/70
Canada	Lic. No. 523

2. Clinical Trials and Studies, Experts' Opinions.

1) Statement on the application of KRYOBULIN™ 250 and 500 units of Factor VIII at the First Medical University Clinic and several other hospitals in Vienna.

a) German Original with signature of Primarius Univ. Doz. Dr. M. Fischer, Head of Control Laboratory of the Krankenhaus der Stadt Wien, Lainz, duly legalized by a Notary Public.

b) Translation of German original into English (Enclosure 7)

2) Excerpt in English from a German original article by M.Fischer, A.Zängl, P.H.Clodi, H.Karobath and K.Lechner on the application of KRYOBULIN™ during Billroth's Operation (B.II) in cases of haemophilia A (Factor VIII Deficiency).

   a) Original full length article in German: "Magenresektion (B.II) bei Hämophilie A (Faktor VIII Mangel)" by M.Fischer, A.Zängl, P.H.Clodi, H.Karobath and K.Lechner, appeared in the Zentralblatt für Chirurgie, 91st edition. 1966, No. 48, edited by Johann Ambrosius Berth, Leipzig.

   b) English excerpt of the above. (Enclosure 5)

3) Excerpt in English from a German original article by M.Fischer and A.Zängl (1st Medical University Clinic and 2nd Surgery University Clinic) on the application of KRYOBULIN™ (Anti-haemophilic Globulin 250 and 500 units of Factor VIII) in the treatment of gastrointestinal bleedings in cases of haemophilia.

   a) Original full length article in German: "Zur Therapie Gastrointestinaler Blutungen bei Haemophilie" by M.Fischer and A.Zängl.

   b) English excerpt of the above. (Enclosure 6)

4) Clinical study carried out by Dozent Dr.Helmut Vinazzer, specialist for internal medicine, Linz, Austria on the application of KRYOBULIN™ 500 and 250 units of Factor VIII.

   a) German original

   b) Translation into English (Enclosure 4)

The final composition of KRYOBULIN™ - Human Antihaemophilic Fraction is as follows:

When dissolved in the volume of Water for Injections, B.P., stated on the label, the solution contains not less than 5 units (± 10 %) per ml; the solution also contains not more than 2.5 percent w/v of fibrinogen, not more than 3.0 percent w/v of total protein, not more than 200 milliequivalents of sodium ions per litre and not more than 165 milliequivalents of citrate ions per litre, with the exception of KRYOBULIN™ 500 units of Factor VIII reconstituted with only 20 ml of Water for Injections, which contain up to 3 % w/v of fibrinogen and up to 6 % w/v of total protein.
In fact, the individual strengths of KRYOBULIN™ are composed as follows:

KRYOBULIN™
Human Antihaemophilic Fraction
100 units of Factor VIII
to be reconstituted with 10 ml of Water for Injections B.P.
1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3.0 % w/v
-thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	267.0 m.moles/litre
Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
250 units of Factor VIII
to be reconstituted with 20 ml of Water for Injections, B.P.

1 ml of the solution contains 12,5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v
- thereof Fibrinogen	1 - 1.5 % w/v
Glycine	267.0 m. moles/litre
Na^+	185.0 .eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 20 ml Water for Injections, B.P.

1 ml of the solution contains 25 units (± 10 %) of Factor VIII.

Total protein	4 - 6 % w/v
- thereof Fibrinogen	2 - 3 % w/v
Glycine	267.0 m.moles/litre
Na^+	185.0 eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 40 ml Water for Injections, B.P.

1 ml of the solution contains 12.5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v
-thereof Fibrinogen	1 - 1.5 % w/v
Glycine	267.0 m.moles/litre
Na^+	185.0.eq/litre
Cl^-	103.0 m.eq/litre
$Citrate^{3-}$	82.0 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

500 units of Factor VIII

to be reconstituted with 100 ml Water for Injections, B.P.

1 ml of the solution contains 5 units (± 10 %) of Factor VIII.

Total protein	0.8 - 1.2 % w/v
- thereof Fibrinogen	0.4 - 0.6 % w/v
Glycine	267.0 m.moles/litre
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

1000 units of Factor VIII

to be reconstituted with 100 ml Water for Injections, B.P.

1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3 % w/v
- thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	267.0 m.moles/litre
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

D) Tests performed on the final product.

1) Solubility in water:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

2) Stability:

No formation of Fibrin for at least 30 minutes after reconstitution.

3) Identification:

a) By Precipitation Test:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13. The Test shall be made on the contents of a final labelled container which has been selected at random from the fillings of each lot or portion of a lot. The test includes a positive test for human serum protein and a negative test for any other animal serum protein.

b) By Factor.VIII activity assay as described under item . . . British Pharmacopoeia 1968, Addendum 1971, page 13.

4) Loss on drying:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

5) Test for Freedom from Pyrogenie Substances:

according to the British Pharmacopoeia 1968, page 1348, using 10 units per kg of the rabbit's weight.

6) Test for sterility:

under aerobic and anaerobic conditions.
Number of final containers tested: 20
Quantity per final container tested: 2 ml
Number of culture tubes:
20 x 1 ml on 10 ml fluid Thioglycollate
20 x 1 ml on 10 ml Soya Bean Casein Digest Medium
Number of days incubation: 14 days
Temperature of incubation: Thioglycollate at 32°C Soya Bean Casein Digest Medium at 20 - 25°C.

7) Assay for total protein:

determination of the protein content according to Kjehldahl; nitrogen value multiplied by 6.25.

ENCLOSUREE no. 1 (a)

DRAFT of INNER LABEL

KRYOBULIN™ 100

Human Antihaemophilic Fraction
100 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 10 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre
Citrate^{3-} 82.0 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Expiry Date:

Contains no preservative.

ENCLOSURE no. 1(b)

DRAFT of INNER LABEL

KRYOBULIN™ 250

Human Antihaemophilic Fraction
250 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
Citrate $^{3-}$	82.0 m.eq/litre
Stabilizer:	
Glycine	267.0 m.moles/litre

Expiry Date:

Contains no preservative.

ENCLOSURE no. 1(c)

DRAFT of INNER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 3.0 percent of fibrinogen and not more than 6.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre
Citrate^{3-} 82.0 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Expiry Date:

Contains no preservative.

ENCLOSURE no. 1 (d)

DRAFT of INNER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 40 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre
Citrate^{3-} 82.0 m.eq/litre

Expiry Date:

Stabilizer:
Glycine 267.0 m.moles/litre

Contains no preservative.

ENCLOSURE no. 1(e)

DRAFT of INNER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

*One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:
Expiry Date:
Approx. electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Citrate $^{3-}$ 82 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Contains no preservative.

ENCLOSURE no. 1(f)

DRAFT of INNER LABEL

KRYOBULIN™ 1000

Human Antihaemophilic Fraction
1000 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may accupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.
Batch No.:
Approx.electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Expiry Date: Citrate^{3-} 82 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre
Contains no preservative.

ENCLOSURE no. 3 (a)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 100

Human Antihaemophilic Fraction
100 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 10 ml of Water for Injections. B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

Enclosed: - 10ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Expiry Date:

Approx. electrolyte concentration:

Na^+	185.0 m.eq/litre
Cl^-	103.0 m.eq/litre
Citrate $^{3-}$	82.0 m.eq/litre
Stabilizer:	
Glycine	267.0 m.moles/litre

Contains no preservative.

ENCLOSURE no. 3 (6)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 250

Human Antihaemophilic Fraction
250 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-20 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Expiry Date:

Approx.electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre
Citrate $^{3-}$ 82.0 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Contains no preservative.

ENCLOSURE no. 3(c)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 02.15/0003
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

Enclosed:-20 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6° C., protected from light.

The reconstituted preparation contains not more than 3.0 percent of fibrinogen and not more than 6.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre
Citrate $^{3-}$ 82.0 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Expiry Date:

Contains no preservative.

- ENCLOSURE no. 3 (a)

DRAFT of OUTER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 02.15/0003
To be reconstituted with 40 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-40 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:
Na^+ 185.0 m.eq/litre
Cl^- 103.0 m.eq/litre

Expiry Date: Citrate^{3-} 82.0 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Contains no preservative.

ENCLOSURE no. 3(e)

DRAFT of OUTER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-100 ml of Water for Injections, B.P.
- 1 transfer tube
- 1 transfusion set with filter

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Expiry Date:

Approx.electrolyte concentration:
Na^+ 185.0m.eq/litre
Cl^- 103.0m.eq/litre
Citrate $^{3-}$ 82.0m.eq/litre
Stabilizer:
Glycine 267.0 m. moles/litre

Contains no preservative.

ENCLOSURE no. 3 (f)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 1000

Human Antihaemophilic Fraction
1000 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. 0215/0003
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-100 ml of Water for Injections, B.P.
- 1 transfer tube
- 1 transfusion set with filter

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Expiry Date:

Approx. electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Citrate $^{3-}$ 82 m.eq/litre
Stabilizer:
Glycine 267.0 m.moles/litre

Contains no preservative.

Distributed by
SEROLOGICAL PRODUCTS LIMITED
6th Floor, Regina House
5, Queen Street, London EC4N 1SP

Manufactured by
ÖSTERREICHISCHES INSTITUT FÜR
HAEMODERIVATE GES.M.B.H.
Production Division of
IMMUNO AG Vienna Austria

I acknowledge hereby receipt of the following documents:

- APPLICATION FOR THE GRANT OF A PRODUCT LICENCE FOR KRYOBULIN TM - HUMAN ANTIHAEMOPHILIC FRACTION
  + Enclosures no. 1, 2, 3, 4, (drafts of labels and circular)
- SCIENTIFIC EVIDENCE
  + Enclosure no. 1 (Method of Manufacture)
  Enclosure no. 2 (Methods of Analysis)
  Enclosure no. 3 (Stability Report)
  Scientific Articles as mentioned under item 2 of REPORT ON CLINICAL STUDIES AND TRIALS (4 original German articles + translations or exceprts in Englisch).

Documents are handed over as originals. After having made out the necessary amount of copies to be filed with the Ministry of Health, I shall send back the original to IMMUNO A.G., Dptmt. of Registrations.

GRO-C

Mr. Norman Berry,
Managing Director.

Vienna, December 2nd, 1972.

Enclosure no. 4

DRAFT OF CIRCULAR

K R Y O B U L I N™

Human Antihaemophilic Fraction

KRYOBULIN™ is a Factor VIII concentrate derived from fresh human plasma, and is indicated for use in the treatment of all coagulation disorders caused by Factor VIII deficiency.

MANUFACTURE AND COMPOSITION

KRYOBULIN™ is prepared from the pooled plasma of healthy donors and freeze-dried for stabilization. All donors, whose plasma is used for the production of KRYOBULIN™, are tested at each donation for their GPT level and the absence of AU/SH/HA antigens (Hepatitis Associated Antigen). Any donor, who has a history of a pathological transaminase level or a positive AU/SH/HA antigen test, is permanently excluded from the donor programme. Despite these precautions, the risk of transmission of homologous serum hepatitis can only be diminished, and not completely eliminated.

INDICATIONS

Ref. 1,2,3,4,5,6,7,8,9,10,11,12,13,16,18,19,22,23,24,28,29,30

Treatment in cases of:

- Haemophilia A
- von Willebrand's disease
- Haemophilia caused by Factor VIII inhibitors
- Thrombocytopenia with decreased Factor VIII activity
- Combined coagulation disorders, also including a reduced Factor VIII activity (consumption coagulopathy, autoimmune diseases, neoplasms, etc.)

Haemorrhages, caused entirely by a Factor VIII deficiency, can be arrested with adequate quantities of KRYOBULIN™. Under controlled therapy with KRYOBULIN™, major surgery (abdominal surgery and orthopaedic surgery) can be performed on patients with a Factor VIII deficiency, even on severe haemophilic patients.

ADMINISTRATION

KRYOBULIN™ must be dissolved immediately before administration using the solvent provided.

a) KRYOBULIN™ 100, 250, and 500 units* (injection) of Factor VIII

After sterilisation of the rubber cap, the solvent is drawn up through one of the three enclosed disposable needles with the attached disposable syringe. After sterilisation of the rubber cap of the bottle containing the lyophilized substance and after insertion of the second disposable needle, the lyophilisate is carefully dissolved. This is best done by very gently agitating the bottle thus avoiding frothing. After reconstitution, KRYOBULIN™ is drawn into the filter-fitted syringe. After removal of this filter and attachment of the third disposable needle, it is administered by slow intravenous injection (over a period of about 10 minutes).

b) KRYOBULIN™ 1000 and 500 units* (transfusion) of Factor VIII

After sterilisation of the rubber caps of both the puncture bottle containing the lyophilized substance and the one containing the solvent, insert the transfer tube into the bottle containing the solvent. Then, lift the puncture bottle containing the lyophilized substance with the bottle-neck held downwards and insert the transfer tube. Subsequently, change the position of the two bottles, with the solvent now being on top and ready to flow into the bottle with the lyophilisate. Remove the transfer tube and gently agitate the bottle. After complete dissolution of the lyophilisate, connect the transfusion set.

DOSAGE AND INDICATIONS Ref. 1,6,8,9,10,11,13,14,15,17,19,24,25,28,29,30

The amount of KRYOBULIN™ which is necessary to be administered may vary considerably according to the response of the individual. As a simple rule to achieve an increase in the Factor VIII concentration of one percent, it is necessary to administer one unit of Factor VIII per kilogramme of bodyweight.

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* One unit of Factor VIII is equivalent to the Factor VIII activity of one ml of average, fresh citrated normal plasma.

Initial treatment requires doses at shorter intervals than maintenance therapy, because of excessive Factor VIII consumption and replenishment of the extravascular compartment. The effectiveness of treatment should be controlled by a Factor VIII assay as partial ~~prothrombin~~ thromboplastin time results in a less accurate value when large quantities of KRYOBULIN™ are being used. If large quantities are used volume overloading may arise and partial removal of patient's plasma by plasmapheresis should be considered.

Bleeding from skin, nose and oral mucous membrane

The initial dose should be 10 units of Factor VIII per kg of bodyweight followed by a maintenance dose of 5 to 10 units of Factor VIII per kg of bodyweight at 6 to 12-hourly intervals.

Haemarthrosis

Approximately 10 units of Factor VIII per kg of bodyweight should be given as an initial dose. The maintenance dose should be 5 to 10 units of Factor VIII per kg of bodyweight at 6 to 12-hourly intervals. Combined with immobilization of the affected joint for several days, the treatment should be sufficient to restore function.

Bruising

In most cases, a single dose of 10 units of Factor VIII per kg of bodyweight is sufficient. With widespread bruising, repeated administration at 6 to 12-hourly intervals, of 5 to 10 units of Factor VIII per kg bodyweight may be required.

Heavy bleeding into muscles

Treatment should be started as soon as possible, since such bleeding may lead to permanent deformity and loss of function. Initial immobilization of the affected area is important. The initial dose ranges from 15 - 20 units of Factor VIII per kg of bodyweight followed by 10 units of Factor VIII per kg of bodyweight at 6-hourly intervals from the first to second day and at 12-hourly intervals from the third to the fifth day.

Haematuria

An initial dose of 15 - 20 units of Factor VIII per kg of bodyweight will be sufficient. For maintenance, 10 units of Factor VIII per kg of bodyweight should be given at 12-hourly intervals.

Major surgery on haemophilic patients

For initial treatment, the administration of at least 25 to 50 units of Factor VIII per kg of bodyweight is recommended. The maintenance dose should be 20 to 40 units per kg of bodyweight starting at 4-hourly intervals from the first to fourth day and at 8-hourly intervals from the fifth to eighth day and later, at 12-hourly intervals until all wounds are healed.

The effect of treatment must be checked daily. Factor VIII activity should not fall below 50 % of the normal average value of 100 %. It is important that treatment should be continued for a sufficient length of time, since the risk of a haemorrhage persists until all wounds are completely healed. Besides the repeated control of Factor VIII, tests for occasionally developing Factor VIII inhibitors should also be carried out on the patient's plasma.

Dental extractions

The amount of Factor VIII to be infused depends on the number and type of teeth to be extracted and on the severity of the haemophilia.

Extraction of one or two teeth

If one or two teeth are to be extracted from a patient suffering from severe haemophilia, 10 to 20 units of Factor VIII per kg of bodyweight should be administered initially. Treatment is continued at 6-hourly intervals from the first to third day, and at 8-hourly intervals from the fourth to the eight day after the extractions.

Extractions of more than two teeth from patients suffering from severe haemophilia

In such cases, a minimum dose of 20 - 30 units of Factor VIII per kg of bodyweight should be given. Maintenance therapy should consist of doses of 10 to 20 units of Factor VIII per kg of bodyweight given in 6-hourly intervals from the first to the third day and in 8-hourly intervals for twelve more days. It is important that the plasma concentration of Factor VIII should not drop below 10 %.

PRECAUTIONS

Though the danger of volume overloading is small with the use of KRYOBULIN™, in cases of major surgery, the control of the patient's central venous pressure, blood pressure and chest-X-ray should be carried out repeatedly as required. If symptoms of volume overloading become apparent, therapeutic plasmapheresis is recommended.

In patients suffering from consumption coagulopathy with a significantly low Factor VIII level, intravascular coagulation must be interrupted by the administration of HEPARIN before the therapy with KRYOBULIN™ is started.

SIDE EFFECTS

Side effects are rarely observed during treatment with KRYOBULIN™ though the following reactions may occur:

1) Allergic Reactions:

All forms of allergic reactions from mild and temporary urticarious rashes to severe anaphylactic shock are possible when human plasma derivatives are administered. If these occur, treatment with KRYOBULIN™ must be interrupted at once. Allergic reactions should be controlled with antihistamines and glucocorticoids and routine shock-treatment given for anaphylactic shock. Careful and frequent recording of pulse rate and blood pressure is essential. If the pulse rate increases and/or the blood pressure falls a transfusion of 5 % Dextrose should be started.

2) Despite the precautions taken in the selection of donors, the risk of transmission of homologous serum hepatitis cannot be entirely excluded when administering human coagulation factors.

3) During every type of therapy involving blood or Factor VIII concentrates, the appearance of a circulating Factor VIII inhibitor is possible. The time at which such an inhibitor is produced cannot be predicted and neither depends on the amount of Factor VIII administered nor on the frequency of administration. According to present experience, the application of corticosteroids or immunosuppressive substances has hardly influenced the formation of inhibitors.

SHELF LIFE AND STORAGE

One and a half years when stored between 2° and 6°C., protected from light.

PACKS

1) KRYOBULIN™ (injection)
   - 1 puncture bottle containing lyophilized KRYOBULIN™ equivalent to 100, 250 or 500 units of Factor VIII,
   - 1 puncture bottle containing Water for Injections, B.P. -
     10 ml for KRYOBULIN™ 100 units of Factor VIII
     20 ml for KRYOBULIN™ 250 units of Factor VIII
     20 ml or 40 ml for KRYOBULIN™ 500 units of Factor VIII
   - 1 disposable syringe
   - 1 filter
   - 3 disposable needles.

2) KRYOBULIN™ (transfusion)
   - 1 transfusion bottle containing lyophilized KRYOBULIN™ equivalent to 500 or 1000 units of Factor VIII
   - 1 puncture bottle containing Water for Injection, B.P.,
     100 ml for KRYOBULIN™ 500 units of Factor VIII
     100 ml for KRYOBULIN™ 1000 units of Factor VIII
   - 1 transfer tube
   - 1 transfusion set with filter

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SEROLOGICAL PRODUCTS LIMITED
6th Floor, Regina House
5,Queen Street, London EC4N 1SP,

Manufactured by
ÖSTERREICHISCHES INSTITUT FÜR
HAEMODERIVATE GES.M.B.H.

Production Division of
IMMUNO AG Vienna Austria

AN APPLICATION FOR THE GRANT

OF A PRODUCT LICENCE

FOR

K R Y O B U L I N [TM]

HUMAN ANTIHAEMOPHILIC FRACTION

SCIENTIFIC EVIDENCE

November 1972

Section A

1. NAMES

Approved Name

HUMAN ANTIHAEMOPHILIC FRACTION

Approved list of the British Pharmacopoeia, Addendum 1971

Monograph Name

HUMAN ANTIHAEMOPHILIC FRACTION

British Pharmacopoeia, Addendum 1971, page 12.

U.S. Adopted Name

Antihaemophilic Globulin (Human).U.S. Minimum Requirements, published by the U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, Maryland, January6, 1947.

Antihaemophilic Factor (Human). TENTATIVE TECHNICAL STANDARDS, National Institutes of Health, Bethesda, Maryland 20014, January 25, 1966, revised: September 22, 1967 and ADDITIONAL STANDARDS: ANTIHAEMOPHILIC FACTOR (HUMAN) (Draft) as amending PART 73, Title 42, of the PUBLIC HEALTH SERVICE REGULATIONS for the manufacture of Biological Products, U.S. Department of Health, Education, and Welfare, Public Health Service, revised: June 1, 1971.

International Non-Proprietary Name

Antihaemophilic Globulin (Human). Antihaemophilic Factor (Human). Factor VIII Concentrate.

Laboratory Code

Nil.

Chemical Name

Not applicable.

Proprietary or Trade Name

Currently marketed in Europe (mainly Austria, West-Germany and Italy) and overseas by the manufacturers, ÖSTERREICHISCHES INSTITUT FÜR HAEMODERIVATE GES.M.B.H. - Production Division of IMMUNO AG Vienna, Austria, under the trade name of KRYOBULIN™. Proposed proprietary or trade name in the United Kingdom is KRYOBULIN™- Human Antihaemophilic Fraction.

2. DESCRIPTION

KRYOBULIN™ - Human Antihaemophilic Fraction is freeze-dried for stability in storage. The lyophilized substance is a white to yellowish amorphous powder or friable solid. After reconstitution, a clear or slightly opalescent colourless solution results.
The product is available in the following concentrations:

1) Injections FOR SOLUTION IN

KRYOBULIN- . . . . . 100 units* of Factor VIII (10 ml)
KRYOBULIN- . . . . . 250 units* of Factor VIII (20 ml)
KRYOBULIN- . . . . . 500 units* of Factor VIII (20 ml)
KRYOBULIN- . . . . . 500 units* of Factor VIII (40 ml)

2) Transfusions

KRYOBULIN-. . . . . 500 units* of Factor VIII (100 ml)
KRYOBULIN-. . . . . 1000 units* of Factor VIII (100 ml)

KRYOBULIN™-Human Antihaemophilic Fraction is a preparation of human blood as defined in Regulation 2 (1) of Statutory Instruments 1963, No. 1456 - The Therapeutic Substances (Manufacturers of Preparations of Human Blood) Regulations 1963.

KRYOBULIN™ meets the requirements set forth in the British Pharmacopoeia 1968 page 116, items (a) to (d) concerning Whole Human Blood and complies also with the monograph for Human Antihaemophilic Fraction included in the Addendum 1971 to the British Pharmacopoeia.

*One unit of Factor VIII is equivalent to the Factor VIII activity of one millilitre average normal citrated plasma.

3. METHOD OF MANUFACTURE

A detailed report on manufacture of KRYOBULIN™ as well as a flow-sheet elaborated by the manufacturers is attached as enclosure no.1

4. IMPURITIES

See quality control tests.

5. DEVELOPMENT CHEMISTRY

Not applicable.

6. SPECIFICATION

A detailed report on the Methods of Analysis employed by the manufacturer for KRYOBULIN™ is attached as Enclosure no. 2.

7. BATCH ANALYSES

Each batch released by the manufacturer is submitted to the quality control tests given below:

7.1 Batch number:

09M0672

7.2 Batch sizes:

09M0672-43 x 20 ml containing 250 units of Factor VIII each.

7.3 Date of manufacture

09M0672-29.8.72

7.4 Results from each test applied, included in the Methods of Analyses - item 6.

Solubility in water:	Batch no. 09M0672 completely soluble in 20 ml solvent
Stability:	does not show any formation of fibrin during 30 minutes.
Factor VIII activity:	280 units per final container.
Loss on drying:	0.4 %
Total protein content:	2.9 %
Sterility:	complies
Pyrogens:	complies
AU/SH/HAA:	negative

Identity:	against anti-human serum: positive; against anti-equine, anti-bovine and anti-ovine: below 0.1 %.
Innocuity:	complies

For batches to be imported into the U.K. all tests as specified in the complete Methods of Analysis attached will be carried out before release by the manufacturer.

8) STABILITY REPORTS

Relevant information by the Manufacturer is attached as Enclosure 3).

Section B. FINISHED PRODUCTS

9) FORMULATION

See attached Report on Manufacture provided by the Manufacturer, item 10).

10) METHOD OF MANUFACTURE OF THE DOSAGE FORM

Bulk material is reconstituted, assayed for Factor VIII activity, filtered under aseptic conditions and filled into previously siliconized and sterilized final containers. It is then freeze-dried.

11) QUALITY CONTROL

11.2 to 11.5; see attached Methods of Analysis.

12) STABILITY REPORTS

Relative information by the Manufacturer is attached as Enclosure no.3.

13) PROPOSED SHELF-LIFE FOR THE PRODUCT

One and a half years.

14) CONTAINERS

Glass puncture bottles or glass transfusion bottles with rubber closure held by a metal collar.
Storage: at 2°to 6°C, protected from light.

REPORTS OF EXPERIMENTAL AND BIOLOGICAL STUDIES

ANIMAL TOXICOLOGY

Test for General Safety.

Any batch of KRYOBULIN™ released by the manufacturer is tested for innocuity. The test is carried out according to the U.S. Public Health Service Regulations, para.73.720 as revised by Draft-Tentative Standards of the Division of Biologics Standards, dated October 8,1971.

Subcutaneous injection of 0.5 ml into two mice not exceeding 20 grammes of weight.

Subcutaneous injection of 5.0 ml into at least two guinea pigs not exceeding 400 grammes of weight.

Observation period: 7 days.

Each animal shall be weighed and the weight recorded on at least the first and last day of the test period. Each animal shall be observed for any abnormal characteristics related to the product and the observations recorded at least four times during the test period, including the first and last day.

Test requirements:

A filling meets the requirements for a satisfactory test if all animals

1) survive the test period
2) do not exhibit any abnormal signs during the test period and
3) weigh at seven days no less than the weight at the time of injections.

REPORTS OF CLINICAL TRIALS AND STUDIES

The following information is provided:

1. KRYOBULIN™ was first produced in 1965. Up to 1969 KRYOBULIN™ was sold on the Austrian Market in the following concentrations.

KRYOBULIN™	100 units	of Factor VIII
KRYOBULIN™	250 units	of Factor VIII
KRYOBULIN™	400 units	of Factor VIII
KRYOBULIN™	500 units	of Factor VIII

From 1965 to 1969 a total of 728.500 units of Factor VIII was sold on the Austrian market,

From 1969 onwards KRYOBULIN™ was also exported and sold on foreign markets.

In 1969 a total of 1,362.700 units of KRYOBULIN™ was sold,

1,009.700 units	in Austria
349.000 units	in the Federal German Republic
4.000 units	in Italy and Switzerland

In 1970 a total of 3,475.550 units of KRYOBULIN™ was sold,

1,511.900 units	in Austria
1,754.950 units	in the Federal German Republic
81.250 units	in Italy
127.450 units	in several countries in Europe and overseas

In 1971 a total of 5,794.500 units of KRYOBULIN™ was sold,

2,216.200 units	in Austria
3,199.450 units	in the Federal German Republic
319.100 units	in Italy
169.750 units	in several countries in Europe and overseas

In 1972 a total of 8,712.100 units of KRYOBULIN™ was sold, there of:

1,773.850 units	in Austria
5,524.000 units	in the Federal German Republic
1,201.000 units	in Italy
135.000 units	in Spain
78.250 units	in several countries of Europe and overseas

Winding up, it may be stated that from 1965 up to the present a total of 20,073.350 units of Factor VIII (KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC FRACTION) in the strenghts of 100, 250, 400 and 500 units of Factor VIII per pack have been sold and that up to the present no serious adverse reactions have been reported.

However, the following side effects may occur:

1) Allergic Reactions:
   All forms of allergic reactions from mild and temporary urticarious rashes to severe anaphylactic shock are possible when human plasma derivatives are administered. If these occur, treatment with BEBULIN™ must be interrupted at once. Allergic reactions should be controlled with antihistamines and glucocorticoids and routine shock-treatment given for anaphylactic shock. Careful and frequent recording of pulse rate and blood pressure is essential. If the pulse rate increases and/or the blood pressure falls a transfusion of 5 % Dextrose should be started.

2) Despite the precautions taken in the selection of donors, the risk of transmission of homologous serum hepatitis cannot be entirely excluded when administering human coagulation factors.

3) During every type of therapy involving blood or Factor VIII concentrates, the appearance of a circulating Factor VIII inhibitor is possible. The time at which such an inhibitor is produced cannot be predicted and neither depends on the amount of Factor VIII administered nor on the frequency of administration. According to present experience, the application of corticosteroids or immunosuppressive substances has hardly influenced the formation of inhibitors.

4) When large quantities of KRYOBULIN™ are administered the danger of volume overloading may arise.

A detailed description of treatment, precautions and treatment of any reaction mentioned above is contained in the circular enclosed in each pack sold.

Registration of KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC FRACTION has been applied for at the Health Authorities of the following countries:

Federal German Republic
Italy
Spain
Portugal
Argentine
Brazil
Peru
U.S.A.

KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC FRACTION has been registered with the Health Authorities of the following countries:

Greece	Lic. No. 6 10053/71
Chile	Lic. No. 1.433/70
Canada	Lic. No. 523

2. Clinical Trials and Studies, Experts' Opinions,

1) Statement on the application of KRYOBULIN™ 250 and 500 units of Factor VIII at the First Medical University Clinic and several other hospitals in Vienna.

a) German Original with signature of Primarius Univ. Doz. Dr. M. Fischer, Head of Control Laboratory of the Krankenhaus der Stadt Wien, Lainz, duly legalized by a Notary Public.

b) Translation of German original into English.

2) Excerpt in English from a German original article by M.Fischer, A.Zängl, P.H.Clodi, H.Karobath and K.Lechner on the application of KRYOBULIN™ during Billroth's Operation (B.II) in cases of haemophilia A (Factor VIII Deficiency).

   a) Original full length article in German: "Magenresektion (B.II) bei Hämophilie A (Faktor VIII Mangel)" by M.Fischer, A.Zängl, P.H.Clodi, H.Karobath and K.Lechner, appeared in the Zentralblatt für Chirurgie, 91st edition. 1966, No. 48, edited by Johann Ambrosius Berth, Leipzig.

   b) English excerpt of the above.

3) Excerpt in English from a German original article by M.Fischer and A.Zängl (1st Medical University Clinic and 2nd Surgery University Clinic) on the application of KRYOBULIN™ (Anti-haemophilic Globulin 250 and 500 units of Factor VIII) in the treatment of gastrointestinal bleedings in cases of haemophilia.

   a) Original full length article in German: "Zur Therapie Gastrointestinaler Blutungen bei Haemophilie" by M.Fischer and A.Zängl.

   b) English excerpt of the above.

4) Clinical study carried out by Dozent Dr.Helmut Vinazzer, specialist for internal medicine, Linz, Austria on the application of KRYOBULIN™ 500 and 250 units of Factor VIII.

   a) German original

   b) Translation into English

ENCLOSUREE no. 1 (a)

DRAFT of INNER LABEL

K R Y O B U L I N ™ 100

Human Antihaemophilic Fraction
100 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 10 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:
Na^+ 185 ~~10.5~~ m.eq/litre
Cl^- 103 ~~10.3~~ m.eq/litre
Citrate $^{3-}$ 22 ~~0.2~~ m.eq/litre
Stabilizer:
Glycine 267 ~~2.7~~ m.moles/litre

Expiry Date:

Contains no preservative.

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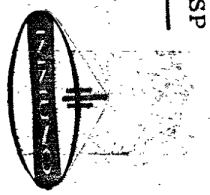
IMMUNO AG Vienna Austria



ENCLOSURE no. 1(b)

DRAFT of INNER LABEL

KRYOBULIN™ 250

Human Antihaemophilic Fraction
250 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:		
Na^+	~~77.0~~ 185	m.eq/litre
Cl^-	~~20.6~~ 103	m.eq/litre
Citrate $^{3-}$	~~16.4~~ 82	m.eq/litre
Stabilizer:		
Glycine	~~5.4~~ 267	m. moles/litre

Contains no preservative.

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Production Division of
IMMUNO AG Vienna Austria

ENCLOSURE no. 1(c)

DRAFT of INNER LABEL

K R Y O B U L I N ™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 3.0 percent of fibrinogen and not more than 6.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	~~37.0~~ m.eq/litre	185
Cl^-	~~20.6~~ m.eq/litre	103
Citrate $^{3-}$	~~16.4~~ m.eq/litre	82
Stabilizer:		
Glycine	~~5.4~~ m.moles/ litre	267

Expiry Date:

Contains no preservative.

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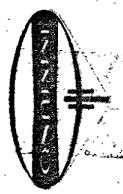

ENCLOSURE no. 1 (d)

DRAFT of INNER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 40 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	~~74.0~~ m.eq/litre	185
Cl^-	~~41.0~~ m.eq/litre	103
Citrate $^{3-}$	~~32.0~~ m.eq/litre	82
Stabilizer:		
Glycine	~~10.7~~ m.moles/litre	267

Contains no preservative.

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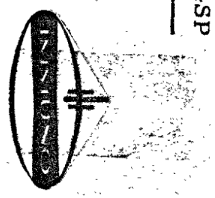

ENCLOSURE no. 1(e)

DRAFT of INNER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:
Approx.electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Expiry Date: Citrate^{3-} 82 m.eq/litre
Stabilizer: ~~27~~ 26 m.moles/litre
Glycine

Contains no preservative.

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ENCLOSURE no. 1(f)

DRAFT of INNER LABEL

K R Y O B U L I N ™ 1000

Human Antihaemophilic Fraction
1000 Units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may accupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.
The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:
Expiry Date:

Approx.electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Citrate $^{3-}$ 82 m.eq/litre
Stabilizer:
Glycine 26.7 m.moles

Contains no preservative.

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ENCLOSURE no. 3 (a)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 100

Human Antihaemophilic Fraction
100 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 10 ml of Water for Injections. B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

Enclosed: - 10ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:
Na^+ ~~185~~ 185 m.eq/litre
Cl^- ~~103~~ 103 m.eq/litre
Citrate $^{3-}$ ~~8.2~~ 8.2 m.eq/litre
Stabilizer:
Glycine ~~2.7~~ 2.7 m.moles/litre

Contains no preservative.

185
103
8.2
2.67

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ENCLOSURE no. 3 (6)

DRAFT of OUTER LABEL

KRYOBULIN™ 250

Human Antihaemophilic Fraction
250 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

Enclosed:-20 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	~~37.0~~ m.eq/litre	185
Cl^-	~~20.6~~ m.eq/litre	103
Citrate^{3-}	~~16.4~~ m.eq/litre	82
Stabilizer:		
Glycine	~~5.4~~ m.moles/litre	267

Expiry Date:

Contains no preservative.

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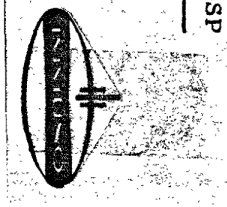

ENCLOSURE no. 3(c)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 20 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-20 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6° C., protected from light.

The reconstituted preparation contains not more than 3.0 percent of fibrinogen and not more than 6.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	~~57.0~~ m.eq/litre	185
Cl^-	~~20.6~~ m.eq/litre	103
Citrate^{3-}	~~16.4~~ m.eq/litre	82
Stabilizer:		
Glycine	~~5.4~~ m.moles/litre	267

Expiry Date:

Contains no preservative.

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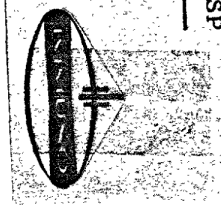

ENCLOSURE no. 3(a)

DRAFT of OUTER LABEL

KRYOBULIN™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 40 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be injected intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-40 ml of Water for Injections, B.P.
- 1 disposable syringe
- 1 filter
- 3 disposable needles

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:

Na^+	~~74.0~~	155 m.eq/litre
Cl^-	~~41.2~~	103 m.eq/litre
Citrate^{3-}	~~32.0~~	82 m.eq/litre
Stabilizer: Glycine	~~10.7~~	267 m.moles/litre

Contains no preservative.

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ENCLOSURE no. 3(e)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 500

Human Antihaemophilic Fraction
500 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . .
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing.
Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution.
Do not use the preparation if a gel forms on reconstitution.

Enclosed:-100 ml of Water for Injections, B.P.
- 1 transfer tube
- 1 transfusion set with filter

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx.electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre

Expiry Date:

Citrate $^{3-}$ 82 m.eq/litre
Stabilizer: 267 ~~27~~ m. moles/litre
Glycine

Contains no preservative.

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Production Division of
IMMUNO AG Vienna Austria

ENCLOSURE no. 3(f)

DRAFT of OUTER LABEL

K R Y O B U L I N ™ 1000

Human Antihaemophilic Fraction
1000 units* of Factor VIII

Lyophilized / Prepared from a plasma pool of 1000 AU/SH/HAA negative donors.
Product Licence No. . . . . .
To be reconstituted with 100 ml of Water for Injections, B.P. previously warmed to 20°-25°C. Agitate gently during reconstitution to avoid frothing. Reconstitution may occupy up to twenty minutes.

The solution must be transfused intravenously immediately after reconstitution. Do not use the preparation if a gel forms on reconstitution.

Enclosed:-100 ml of Water for Injections, B.P.
- 1 transfer tube
- 1 transfusion set with filter

* One unit of Factor VIII is equivalent to the Factor VIII activity of 1 ml average normal plasma.

Store between 2° and 6°C., protected from light.

The reconstituted preparation contains not more than 2.5 percent of fibrinogen and not more than 3.0 percent of total protein.

Batch No.:

Approx. electrolyte concentration:
Na^+ 185 m.eq/litre
Cl^- 103 m.eq/litre
Expiry Date: $Citrate^{3-}$ 82 m.eq/litre
Stabilizer: ~~26.7~~ 26 m.moles / litre
Glycine

Contains no preservative.

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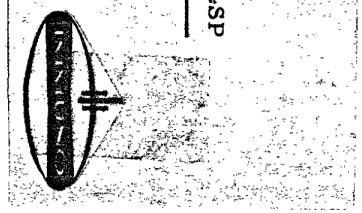
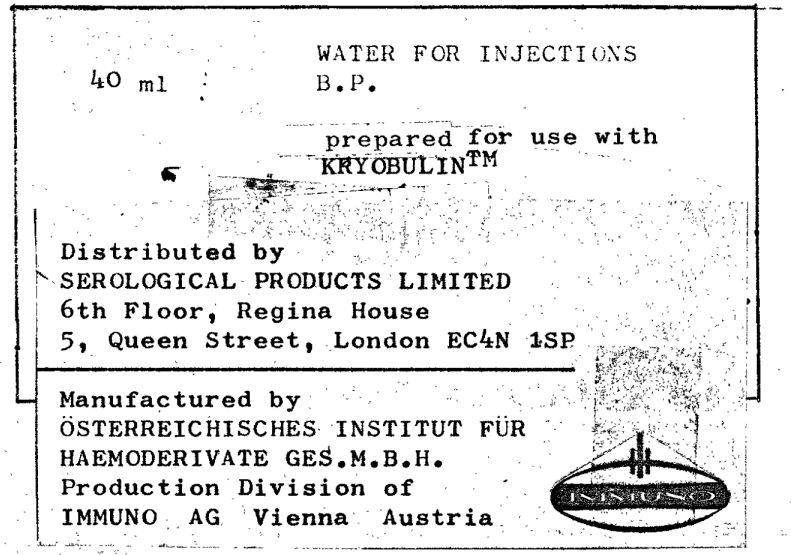


Enclosure no. 2 (a)

DRAFT for SOLVENT CONTAINER LABEL



Enclosure no. 2 (b)

DRAFT for SOLVENT CONTAINER LABEL

100 ml

WATER FOR INJECTIONS
B.P.

prepared for use with
KRYOBULIN™

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5, Queen Street, London EC4N 1SP

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HAEMODERIVATE GES.M.B.H.
Production Division of
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Enclosure no. 2 (c)

DRAFT for SOLVENT CONTAINER LABEL

20 ml

WATER FOR INJECTIONS
B.P.

prepared for use with
KRYOBULIN™

Distributed by
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5, Queen Street, London EC4N 1SP

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Enclosure no. 2 (a)

DRAFT for SOLVENT CONTAINER LABEL

10 ml : WATER FOR INJECTIONS B.P.

prepared for use with KRYOBULIN™

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Enclosure no. 1

ÖSTERREICHISCHES INSTITUT FÜR HAEMODERIVATE

GESELLSCHAFT M. B. H.

PRODUKTIONSBETRIEB DER IMMUNO AKTIENGESELLSCHAFT

1220, INDUSTRIESTRASSE 72, WIEN

METHOD OF MANUFACTURE

1. PRODUCT

KRYOBULIN™ - HUMAN ANTIHAEMOPHILIC GLOBULIN in packs of 100, 250, 500 or 1000 units* of Factor VIII

2. NAME AND ADRESS OF MANUFACTURER

Österreichisches Institut für Haemoderivate Ges.m.b.H.
Industriestrasse 72, 1220 Vienna - Austria

3. PLASMAPHERESIS STATIONS AT

a) Vienna VI, Sandwirtgasse 3,
b) Vienna II, Marinelligasse 15,
c) Linz, Rilkestrasse 20,
d) Graz, Konrad v. Hötzendorfstr. 3,
e) Vöcklabruck, Feldgasse 17,
f) Heidelberg, Bismarckstr. 17, BRD
g) Mannheim, Neckerauerstr. 245 / Mönchwörthstr. 222, BRD

4. SOURCE OF PLASMA

Plasma obtained from human donors aged between 18 and 65 years.

*One unit of Factor VIII is equivalent to the Factor VIII activity of one ml average normal citrated plasma.

TELEPHON 22 15 66 CABEL: HAEMODERIVATE WIEN TELEX: 07-4865 IMMUNO WIEN
CREDITANSTALT-BANKVEREIN KONTO 26-40167, POSTSPARKASSENKONTO 142.655

5. CRITERIA FOR ACCEPTING DONORS PRIOR TO DONATION

Good conditions of health, normal temperature, no increased transaminase values (above 15 I.U. per litre), AU/SH/HAA - negative, cardiolipin negative, (haemoglobin) ~~content 85 % (T.S.A.)~~, and not less than 12.5 % w/v (female donors), or 13.3 % w/v (male donors) B.P., protein at least 6 g%.

6. EQUIPMENT USED FOR PLASMAPHERESIS

Sterile, pyrogenfree disposable plastic bags containing 75 ml Anticoagulant Citrate Dextrose Solution U.S.P. XVIII, Formula A: contains 16.8 m.eq of Sodium.

Each ml contains: 0.8 g Citric Acid (hydrous) U.S.P.
2.2 g Sodium Citrate U.S.P.
2.45 g Dextrose (hydrous) U.S.P.

7. PLASMAPHERESIS

The site over the selected vein is cleaned with a 3 % acetone-76 % ethanol mixture. For each donor a sterile taking set is used. About 500 ml blood are collected. After completion of the bleeding the taking needle remains in the vein; a new transfusion set is adjusted and Ringer solution transfused during the time the blood is centrifuged and the plasma separated from the erythrocytes. The erythrocytes are suspended in Ringer solution and re-transfused to the donor. A precise system including most exact labelling of the bottles and determination of the donor's blood group as well as his erythrocytes suspension before re-transfusion guarantee faultless functioning of plasmapheresis. The process is then repeated.

After separation of plasma and cells, the plasma is centrifuged again for 30 min/2500 rpm and the supernatant is taken off, frozen and stored.

The entire operation is supervised and controlled by a registered medical practitioner.

8. PRECAUTIONS TAKEN DURING PLASMAPHERESIS

A) Only sterile, pyrogenfree, plastic equipment is used and an aseptic procedure is employed in all phases of plasmapheresis.

B) The plastic bag into which the donor's blood is collected shows the donor's name and number. Direct ABO blood grouping is carried out while the blood donation is taken and immediately before infusion of autologous red cells.

C) Approximately 500 ml of plasma are taken per visit.

D) Withdrawn plasma is fresh-frozen and kept in frozen state. During this time several tests, such as Australia Antigen detection, transminase etc., are carried out. Only Australia antigen-free plasma will be used for further processing.

9. METHOD OF PROCESSING

The preparation follows the method of POOL (J.G.POOL and A.E. SHANNON, New Engl.J.Med., 273:1443, 1965). The plasma of the donor is frozen and thawed in the cold. The insoluble cryoprecipitate containing mainly fibrinogen and Factor VIII is centrifuged, the supernatant plasma taken off and the precipitate results. In order to achieve a further specific concentration of Factor VIII, the cryoprecipitate is submitted to further purification. The major part of the non coagulation active fibrinogen is removed by selective elution of Factor VIII. For the purpose of concentration, the eluate is freeze-dried. A Factor VIII activity assay is carried out on the intermediate product. Depending on the results of the same, the intermediate product is reconstituted, the stabilizer added as described under the composition of the product, and the resulting preparation filtered and filled into final containers under sterile conditions. Immediately after filling into the final containers, the final product is freeze-dried for stability in storage and again assayed for Factor VIII activity.

The final composition of KRYOBULIN™ - Human Antihaemophilic Fraction is as follows:

When dissolved in the volume of Water for Injections, B.P., stated on the label, the solution contains not less than 5 units (± 10 %) per ml; the solution also contains not more than 2.5 percent w/v of fibrinogen, not more than 3.0 percent w/v of total protein, not more than 200 milliequivalents of sodium ions per litre and not more than 165 milliequivalents of citrate ions per litre, with the exception of KRYOBULIN™ 500 units of Factor VIII reconstituted with only 20 ml of Water for Injections, which contain up to 3 % w/v of fibrinogen and up to 6 % w/v of total protein.
In fact, the individual strengths of KRYOBULIN™ are composed as follows:

KRYOBULIN™
Human Antihaemophilic Fraction
100 units of Factor VIII
to be reconstituted with 10 ml of Water for Injections B.P.
1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3.0 % w/v
-thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	267 ~~2.7~~ m.moles/litre
Na^+	185 ~~18.5~~ m.eq/litre
Cl^-	103 ~~10.3~~ m.eq/litre
$Citrate^{3-}$	82 ~~8.2~~ m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

250 units of Factor VIII

to be reconstituted with 20 ml of Water for Injections, B.P.

1 ml of the solution contains 12,5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v	
- thereof Fibrinogen	1 - 1.5 % w/v	
Glycine	~~5.4~~ m. moles / litre	267
Na^+	~~37~~ m.eq/litre	185
Cl^-	~~20.6~~ m.eq/litre	103
$Citrate^{3-}$	~~16.4~~ m.eq/litre	82

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

500 units of Factor VIII

to be reconstituted with 20 ml Water for Injections, B.P.

1 ml of the solution contains 25 units (± 10 %) of Factor VIII.

Total protein	4 - 6 % w/v	
- thereof Fibrinogen	2 - 3 % w/v	
Glycine	~~5.4~~ m.moles / litre	267
Na^+	~~37~~ m.eq/litre	185
Cl^-	~~20.6~~ m.eq/litre	103
$Citrate^{3-}$	~~16.4~~ m.eq/litre	82

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION

500 units of Factor VIII

to be reconstituted with 40 ml Water for Injections, B.P.

1 ml of the solution contains 12.5 units (± 10 %) of Factor VIII.

Total protein	2 - 3 % w/v	
-thereof Fibrinogen	1 - 1.5 % w/v	
Glycine	~~10.7~~ m.moles / litre	
Na^+	~~74~~ m.eq/litre	185
Cl^-	~~41.8~~ m.eq/litre	103
$Citrate^{3-}$	~~32.8~~ m.eq/litre	82

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
500 units of Factor VIII
to be reconstituted with 100 ml Water for Injections, B.P.

1 ml of the solution contains 5 units (± 10 %) of Factor VIII.

Total protein	0.8 - 1.2 % w/v
- thereof Fibrinogen	0.4 - 0.6 % w/v
Glycine	~~27~~ m.moles/litre 267
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

KRYOBULIN™
HUMAN ANTIHAEMOPHILIC FRACTION
1000 units of Factor VIII
to be reconstituted with 100 ml Water for Injections, B.P.

1 ml of the solution contains 10 units (± 10 %) of Factor VIII.

Total protein	1.5 - 3 % w/v
- thereof Fibrinogen	0.8 - 1.6 % w/v
Glycine	~~26.7~~ m.moles/litre 267
Na^+	185 m.eq/litre
Cl^-	103 m.eq/litre
$Citrate^{3-}$	82 m.eq/litre

10. METHOD AND TIME OF FILTRATION OF THE SOLUTION

Filtration is carried out with the use of SCHLEICHER & SCHÜLL, BA 83 membrane filter, approx. 30 ml solution per square centimetre per hour.
Pressure: 0.2 kilogramme per square centimetre.

11. CLEANING AND STERILIZING OF FINAL CONTAINERS AND STOPPERS

After cleaning with a solution of detergent at 40° C, glassware is siliconized and subsequently washed with distilled water and sterilized at 180° C for 6 hours. Rubber stoppers etc. are cleaned by the same process and sterilized at 120° C for 2 hours, however without siliconization.

12. FILLING

Semi-automatically under strictly aseptic contitions.

13. FLOW-SHEET

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F L O W - S H E E T

KRYOBULIN™

HUMAN ANTIHAEMOPHILIC FRACTION

Blood donor

↓ bleeding — PLASMAPHERESIS

ACD-blood

↓ 1st centrifugation → Precipitate: cells

Supernatant:
ACD-plasma

↓ 2nd centrifugation → Precipitate: rest of cells (discarded)

Supernatant:
plasma (free of cells)

a) freezing
b) thawing in the cold
c) centrifugation (cold)

↓ → Precipitate:
a) extraction of protein with non-coagulant activity with saline.
b) extraction of Factor VIII containing protein material by saline
c) concentration by freeze-drying resulting "intermediate product"
d) Factor VIII activity assay of "intermediate product".
e) reconstitution of lyophilized "intermediate product"
- adjustment of concentration for the final container
- addition of stabilizers
- adjustment of pH to 6.8 - 7.2
- sterile filtration
- sterile filling into final containers
- freeze drying
f) quality control

Supernatant:
plasma (minus cryoglobulin)

↓

Production of
gamma globulin
and albumin

Re-infusion after dilution with physiological saline (plasmapheresis)

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Enclosure no. 2

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METHODS OF ANALYSIS FOR K R Y O B U L I N™ - Human Antihaemophilic Fraction

A) Tests performed on the donor prior to donation:

1) Haemoglobin value:
   according to the British Pharmacopoeia 1968, page 117.

B) Tests performed on the plasma of each donation:

1) Evidence of syphilitic infection:
   Cardiolipin test.

2) Presence of Australia/Serum Hepatitis/Hepatitis Associated Antigen:
   laboratory own method (crossover electrophoresis)

3) SGPT-activity:
   U.V.Test.

4) Protein content:
   by colorimetric assay (BIURET).

C) Tests performed during manufacture of the product:

1) Assay of Factor VIII activity of freeze-dried "intermediate product"
   Method employed: see Assay of Factor VIII activity on the final product.

2) Determination of pH value:
   according to the British Pharmacopoeia 1968, page 1208.

3) Sterility
   5 ml in 60 ml Fluid Thioglycollate
   5 x 1 ml in 10 ml Fluid Thioglycollate
   Incubation temperature: 32° C
   Incubation time: 14 days

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D) Tests performed on the final product.

1) Solubility in water:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

2) Stability:

No formation of Fibrin for at least 30 minutes after reconstitution.

3) Identification:

a) By Precipitation Test:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13. The Test shall be made on the contents of a final labelled container which has been selected at random from the fillings of each lot or portion of a lot. The test includes a positive test for human serum protein and a negative test for any other animal serum protein.

b) By Factor VIII activity assay as described under item . . . British Pharmacopoeia 1968, Addendum 1971, page 13.

4) Loss on drying:

according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

5) Test for Freedom from Pyrogenie Substances:

according to the British Pharmacopoeia 1968, page 1348, using 10 units per kg of the rabbit's weight.

6) Test for sterility:

under aerobic and anaerobic conditions.

Number of final containers tested: 20

Quantity per final container tested: (1 ml) richtig: 2 ml Kl. Dr. Herrmann

Number of culture tubes:

20 x 1 ml on 10 ml fluid Thioglycollate

20 x 1 ml on 10 ml Soya Bean Casein Digest Medium

Number of days incubation: 14 days

Temperature of incubation: Thioglycollate at 32°C Soya Bean Casein Digest Medium at 20 - 25°C.

7) Assay for total protein:

determination of the protein content according to Kjehldahl; nitrogen value multiplied by 6.25.

8) Assay for total fibrinogen:

will be carried out according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

9) Assay for Sodium ions:

will be carried out according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

10) Assay for citrate ions:

will be carried out according to the British Pharmacopoeia 1968, Addendum 1971, page 13.

11) Assay for potency:

Methods employed

1. Laboratory own method (one stage)

Material: 37°C waterbath, stopwatches, pipettes, glass tubes

Reagents:

a) Factor VIII deficient plasma: Citrated plasma from a patient with severe haemophilia A (factor VIII below 1 %), stored deep frozen.

b) Phospholipid-kaolin suspension: Phospholipid concentrate (Tachostyptan, Hormon Chemie München) is diluted 1:200 in Owren's buffer (11.75 g sodium dietylbarbiturate and 14.67 g sodium chloride in 1570 ml distilled water plus 430 ml 0.1 n hydrochloric acid; pH 7.35). Kaolin is added to a concentration of 0.5 % w/v.

c) Dialysed aged EDTA plasma as diluent for samples:

Nine parts blood from a healthy donor are mixed with one part 40 mM disodium ethylendiaminetetraaceticacid (Na_2EDTA) containing 0.7 % NaCl. The resulting plasma is incubated at 37°C for 48 hours and then dialysed against 0.4 % trisodium citrate.. 5 1/2 H_2O / 0.7 % NaCl for 48 hours.

Storage: deep frozen in portions.

d) m/20 calcium chloride

Procedure:

The reagents (during testing stored in an icebath) are pipetted into glass tubes in the following way:

0.1 ml factor VIII deficient plasma
0.1 ml phospholipid-kaolin suspension
0.1 ml sample (serial dilutions of testing sample: factor VIII concentrate or normal plasma pool respectively).
five minutes incubation at 37°C
0.1 ml m/20 calcium chloride

The time from the addition of calcium chloride until clot formation is measured with a stopwatch.

Calculation of factor VIII concentration:

A standard plasma is prepared by pooling plasma samples from at least 15 healthy donors. A calibration curve is prepared by plotting the clotting times of serial plasma dilutions of this plasma pool (conc., 1:2, 1:4 .....) against the respective concentrations on a double logarithmic paper.

The factor VIII concentrations of the testing sample dilutions are expressed as a percentage of Factor VIII in normal plasma by using the calibration curve. The amount of factor VIII in one bottle of concentrate is calculated by the following formula:

$$\text{Units factor VIII} = \frac{(\%\ \text{Factor VIII}) \times (\text{volume})\ (\text{ml})}{100}$$

1 unit factor VIII is equivalent to the factor VIII activity of 1 ml average fresh citrated plasma.

2. Biological Assay of Human Antihaemophilic Fraction, Addendum 1971, page 120, to the British Pharmacopoeia 1968.

12) Test for Innocuity:

Test for General Safety, paragraph 73.720 of the U.S. Public Health Service Regulations, Title 42, Part 73 revised May 1968 and Draft of Tentative Standards for Informed Review, Revision of General Safety Test dated 8. 10. 1971 issued by the Division of Biologics Standards, U.S. Public Health Service, National Institutes of Health, Bethesda, Md., U.S.A.

Subcutaneous injection of 0.5 ml into two mice not exceeding 20 grammes of weight.

Subcutaneous injection of 5.0 ml into at least two guinea pigs not exceeding 400 grammes of weight.

Observation period: 7 days.

The product passes the test if all animals:

1) survive the test period
2) do not exhibit any abnormal signs during the test period
3) weigh at seven days not less than at the time of injections.

ÖSTERREICHISCHES INSTITUT FÜR HAEMODERIVATE GES.M.B.H.

GRO-C

Vienna, December 1st, 1972

Dr. Otto F. Schwarz

ÖSTERREICHISCHES INSTITUT FÜR HAEMODERIVATE

GESELLSCHAFT M. B. H.

PRODUKTIONSBETRIEB DER IMMUNO AKTIENGESELLSCHAFT

1220, INDUSTRIESTRASSE 72, WIEN

Enclosure no. 3

STABILITY REPORT
for
K R Y O B U L I N™
Human Antihaemophilic Fraction

1) Batch number: 0938770/2
   Date of manufacture: 24.9.1970
   Storage: below 6°C

2) Control assays for Factor VIII activity during storage have been currently carried out.
   Assay methods emplayed:
   a) THROMBOPLASTIN GENERATION TEST
      according to D.ARONSON, M.D., U.S.Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, Rockville, Md.
   b) Laboratory own (IMMUNO) method (1 stage method).
      Results obtained: during a period of 2 years no change of Factor VIII activity occurred.

3) Presence of degradation products after 2 years of storage:
   a) Method employed:
      Microzone electrophoresis (Beckman).
   b) Results obtained:
      the electrophoretic composition of the product remains unchanged, no sign of denaturated protein.

4) Physical characteristics:
   remain unchanged during storage.

5) Nature of containers used for storage:
   Siliconized, sterilized glass bottles.

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Vienna, 30th November 1972

Dr. O.F. Schwarz

TELEPHON 22 15 66 CABEL: HAEMODERIVATE WIEN TELEX: 07-4865 IMMUNO WIEN
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Ergebnisse der Testung von Kryobulin 500 bezw. Kryobulin 250

1. Beschreibung

Kryobulin 500 bezw. 250 (Hersteller: Immuno- A.G., Wien) wird als lyophilisierte, in Glasflaschen abgefüllte Trockensubstanz geliefert. In der Packung befindet sich als Lösungsmittel 100 ml steriles destilliertes Wasser,ausserdem wird ein Einmalgerät zur Infusionen beigegeben.

2. Untersuchungen in vitro

Die Substanz benötigt zur Lösung in 100 ml Wasser bei Zimmertemperatur etwa 20 min. Nach Auflösung von Kryobulin 500 in 100 ml Wasser wurden folgende Werte der Faktor VIII-Aktivität und folgende Fibrinogenkonzentrationen gefunden:
(Die Untersuchungen erfolgten für Faktor VIII mit einer Einstufenmethode,wobei als Substrat natürliches Faktor VIII-Mangelplasma diente, im PTT- System, für Fibrinogen mit einer photometrischen Methode nach Umwandlung des Fibrinogens in Fibrin und Auflösung in kochender 1,5m-Natronlauge. Die Werte für Faktor VIII entsprechen dem Durchschnitt aus je 6 Bestimmungen, für Fibrinogen dem Durchschnitt aus Doppelwerten).

Faktor VIII (% der Norm)	Fibrinogen mg%
490	1432
515	1387
478	1408
462	1455
534	1286
482	1421
512	1272
467	1358
488	1471
521	1395
494,9 ± 36	1388,5 ± 99

Aufgrund dieser Untersuchungen enthält eine Packung Kryobulin 500 etwa die angegebene Menge Faktor VIII sowie zusätzlich ca 1,4 g Fibrinogen.

Univ.-Dozent
Dr. Helmut Vinazzer
Facharzt für Innere Medizin
A-4020 Linz/Donau
Untere Donaulände 12, Tel. 24197

3. Untersuchungen an Patienten

3.1. Fallzahl und Applikation

Vom 1.1.1969 bis 30.6.1972 erhielten insgesamt 20 Patienten mit Hämophilie A (12 schwer, 5 mittelschwer, 3 mild) zusammen 415 Packungen Kryobulin 500 und 63 Packungen Kryobulin 250. Die Applikation erfolgte als intravenöse Infusion. Bei Substitution über einen längeren Zeitraum,etwa nach chirurgischen Eingriffen,wurde ein zentraler Venenkatheter angelegt,durch den die Infusionen erfolgten. In drei Fällen wurde Kryobulin mit einer Motorspritze kontinuierlich in den zentralen Venenkatheter infundiert.

3.2. Ursachen für die Substitutionsbehandlung

Operation eines grossen Pseudotumors	1
traumatische Amputation eines Oberschenkels	1
Fingeramputation	1
Cerebrale Blutung	2
stark blutende Verletzungen	9
Hämaturie	14
Hämarthros	37
Zahnextraktionen	12
Tibiafraktur	1

3.3. Recovery von Faktor VIII

Bei 3 Patienten wurde eine Berechnung der recovery von Faktor VIII durchgeführt. Es handelte sich um drei Fälle von schwerer Hämophilie mit einem Faktor VIII unter 1%.

Fall 1:
Plasmamenge 3160 ml, F. VIII 0,3%
Infusion von 4 Kryobulin 500
in je 60 ml Flüssigkeit
nach Infusion:
Plasmamenge 3400 ml
erwarteter Faktor VIII : 59%
gemessener Faktor VIII: 40%
recovery: 67,5%

Fall 2:
Plasmamenge 1140 ml
Faktor VIII 0,1%
nach 1 Kryobulin 500
in 60 ml Flüssigkeit
Plasmamenge 1200 ml
erwarteter Faktor VIII: 42%
gemessener Faktor VIII: 28%
recovery: 66,6%

Fall 3:
Plasmamenge 1580 ml, F. VIII 0,5%
nach 2 Kryobulin 500 Plasmamenge 1700 ml
erwarteter Faktor VIII 59%
gemessener Faktor VIII 38%
recovery: 63%

Univ.-Dozent
Dr. Helmut Vinazzer
Facharzt für Innere Medizin
A-4020 Linz/Donau
Untere Donaulände 12, Tel 24197

Die Berechnung der recovery ergab bei Erstinfusion Werte zwischen 63 und 67,5%. Sie entspricht also der in der Literatur als Durchschnittswert angegebenen recovery von etwa 2/3.

3.4. Wirkung auf die Hämostase

Bei der Operation des angegebenen Pseudotumors des linken Oberschenkels bei einem 16-jährigen, 62 kg schweren Patienten wurden in der ersten postoperativen Woche täglich 4 Packungen Kryobulin 500 als kontinuierliche Infusion gegeben. Der Faktor VIII betrug während dieser Zeit bei Kontrollen alle 12 Stunden zwischen 50 und 32%. In der zweiten Woche wurden täglich 3 Kryobulin 500 infundiert,die die Faktor VIII-Konzentration bei täglichen Kontrollen auf Werten zwischen 20 und 28% hielten. Die Operation und die postoperative Phase verliefen ohne Blutungszwischenfall.

Bei dem Patienten mit der traumatischen Oberschenkelamputation(nach einem Eisenbahnunglück) war die Hämophilie vorher nicht bekannt. Erst aufgrund einer schweren Blutung wurde ein Gerinnungsstatus durchgeführt,der eine milde Hämophilie A mit einem Faktor VIII von 9% ergab. Bei diesem Patienten wurden täglich 3 Packungen Kryobulin 500 durch 2 Wochen gegeben. Es handelte sich ebenfalls um einen Erwachsenen mit einem Gewicht von 72 kg. Die Faktor VIII-Werte bewegten sich bei täglichen Kontrollen zwischen 37 und 18%. Die Blutung konnte prompt zum Stillstand gebracht werden. Die grösseren Faktor VIII-Schwankungen sind dadurch zu erklären,dass bei diesem Patienten Kryobulin nicht als kontinuierliche Infusion, sondern in 3 täglichen Infusionen gegeben wurde.

Auch bei dem Patienten,dem ein Finger amputiert wurde,war vorher nichts von der Hämophilie bekannt. Erst nach einer Nachblutung,die bereits eine Woche dauerte,wurde ein Gerinnungsstatus angefordert,der eine milde Hämophilie A mit einem Faktor VIII von 6% ergab. Nach täglicher Infusion von 3 Packungen Kryobulin 500 wurden Werte von 30 bis 36% Faktor VIII festgestellt und die Blutung sistierte. Die Therapie wurde durch 2 Wochen fortgesetzt.

Zu einem prompten Sistieren der Blutung kam es auch bei den

Univ.-Dozent
Dr. Helmut Vinazzer
Facharzt für Innere Medizin
A-4020 Linz/Donau
Untere Donaulände 12, Tel. 24197

cerebralen Blutungen sowie bei den weniger dramatischen Hämorrhagien wie Gelenksblutungen,Hämaturien, Hämatomen, und Frakturen. Bei Zahnextraktionen wurde ebenfalls grundsätzlich eine Substitution mit Kryobulin durchgeführt,wobei eine Faktor VIII-Konzentration von 20% durch eine Woche angestrebt wurde.

Die Dosierung wurde individuell ermittelt und richtete sich immer nach der Höhe des angestrebten Faktor VIII. Nur bei Hämarthros schon bekannter Patienten,bei denen es sich gewöhnlich um Kinder zwischen 3 und 12 Jahren handelte,wurden 250 bis 750 Einheiten Kryobulin innerhalb von 24 Stunden verabreicht. Dabei erfolgte gewöhnlich nur eine Bestimmung der PTT vor und in Abständen von 12 - 24 Stunden nach der Behandlung.

Von den 20 Patienten erhielt innerhalb der angegebenen Zeit 1 Patient 115 Packungen Kryobulin 500, ein weiterer 58, 2 Patienten je 27, die übrigen zwischen 23 und 5 Packungen. Die bisher niedrigste Dosierung bei einem 1968 geborenen Knaben betrug insgesamt 6 Packungen Kryobulin 250 bei vier Recidiven von Hämarthros.

3.5. Nebenwirkungen

In keinem der beschriebenen Fälle trat eine Hypervolämie als Folge einer massiven Substitutionstherapie auf. Allerdings war das grösste verabreichte Flüssigkeitsvolumen 700 ml in 24 Stunden bei einem Erwachsenen. Da in keinem der Fälle ein Hemmkörper gegen Faktor VIII auftrat,war eine massivere Substitution,bei der eine Hypervolämie befürchtet werden müsste, nicht erforderlich.

An Nebenwirkungen sind aufgetreten:

Schüttelfrost mit Temperaturanstieg über 39°	1
anaphylaktische Reaktionen	0
Hämolysezeichen	0
Thrombopenie,Verbrauchskoagulopathie	0
Hepatitis	0

Zur Feststellung einer latenten Hepatitis wurden bei 14 Patienten in Abständen von 2 bis 4 Wochen bis zu 6 Monaten nach

Univ.-Dozent
Dr. Helmut Vinazzer
Facharzt für Innere Medizin
A-4020 Linz/Donau
Untere Donaulände 12, Tel 24197

der Substitution klinische Kontrollen sowie Untersuchungen vonSGOT, SGPT, LDH, Serumbilirubin und Urobilinogen im Harn durchgeführt. In keinem der Fälle ergab sich ein Hinweis auf eine manifeste oder latente Hepatitis.

Linz, den 20.11.1972

Univ.-Dozent
Dr. Helmut Vinazzer
Facharzt für Innere Medizin
A-4020 Linz/Donau
Untere Donaulände 12, Tel. 24197

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Blutgerinnungslaboratorium
Univ.Doz.Dr.Helmut Vinazzer
A- 4020 Linz, Österreich
Untere Donaulände 12

TRANSLATION

RESULTS OF A CLINICAL STUDY USING KRYOBULIN 500 AND KRYOBULIN 250

1. Description

KRYOBULIN 500 and KRYOBULIN 250 (manufacturer: IMMUNO AG Vienna) is distributed as a lyophilized substance filled into glass bottles. Each pack of KRYOBULIN includes 100 ml of sterile, distilled water for reconstitution as well as one disposable transfusion set.

2. In vitro tests

The substance takes about 20 minutes for complete reconstitution in 100 ml of solvent at room temperature. After reconstitution of KRYOBULIN 500 units in 100 ml of solvent, the solution was assayed for Factor VIII activity and fibrinogen concentration. The following values resulted:

(Determination of Factor VIII was carried out by a one stage method in the PTT system with a Factor VIII deficient plasma from natural source serving as substrate. The fibrinogen values were ascertained with the aid of a photometric method after transformation of fibrinogen into fibrin and dissolving the same in boiling 1.5 m sodium hydrate (NaOH). The values of Factor VIII activity correspond to the average values of six determinations and of fibrinogen to the average value of two assays.

Factor VIII (% of the standard)	Fibrinogen mg %
490	1432
515	1387
478	1408
462	1455
534	1286
482	1421
512	1272
467	1358
488	1471
521	1395
494,9	1388,5 ± 99

The results of these tests proved that a pack of KRYOBULIN 500 approximately contains the amount of Factor VIII mentioned on the label as well as, in addition, 1,4 grammes of fibrinogen.

3. In vivo tests

3.1 Number of patients and administration:

Between January 1st, 1969 and June 30, 1972 20 patients with haemophilia A (12 severe, 5 moderate, 3 mild cases) received a total of 415 packs of KRYOBULIN 500 and 63 packs of KRYOBULIN 250. As route of administration, intravenous transfusion was used. In case of treatment over a longer period of time, e.g. after surgical interventions, transfusion was done by a central venous catheter. In the case of three patients, KRYOBULIN was transfused continuously by a motor syringe into the central venous catheter.

3.2 Indications for treatment

- operation of a big pseudo tumor	1
- traumatic amputation of a thigh	1
- amputation of a finger	1
- cerebral haemorrhages	2
- severly bleeding injuries	9
- haematuria	14
- haemarthroses	37
- dental extractions	12
- fracture of the tibia	1

3.3. In vivo recovery of Factor VIII

In vivo recovery of Factor VIII was calculated in three patients, who were all severe cases of haemophilia with a Factor VIII activity below 1 %.

Case 1:
Volume of plasma 3160 ml, Factor VIII 0.3 %
Transfusion of 4 x KRYOBULIN 500 in
60 ml of solvent each.
After transfusion:
Volume of plasma 3400 ml
Factor VIII activity expected: 59 %
Factor VIII activity found: 40 %
Recovery: 67.5 %

Case 2:
Volume of plasma 1140 ml
Factor VIII activity 0.1 %
Transfusion of 1 pack of KRYOBULIN 500
in 60 ml solvent.
Volume of plasma 1200 ml
Factor VIII activity expected: 42 %
Factor VIII activity found: 28 %
Recovery: 66.6 %

Case 3:
Volume of plasma 1580 ml, Factor VIII 0.5 %
Transfusion of 2 packs of KRYOBULIN 500
Volume of plasma 1700 ml
Factor VIII activity expected: 59 %
Factor VIII activity found: 38 %
Recovery: 63 %

The values of recovery calculated ranged between 63 and 67.5 % in case of primary transfusions. As a consequence recovery values obtained correspond to the average values of 2/3 given in pertinent scientific literature.

3.4 Effect on haemostasis

When operating on the pseudotumor of the left thigh in a 16 year old patient weighing 62 kgs, 4 packs of KRYOBULIN 500 were administered daily as a permanent infusion during the first postoperative week. Throughout this period, Factor VIII activity was assayed each 12 hours and found to be between 50 and 32 %. During the second week a daily dose of 3 packs of KRYOBULIN 500 was transfused maintaining Factor VIII concentration between 20 and 28 % at daily controls. Both, operation and post-operative course were without any bleeding incident.

Haemophilia was not known in the patient with the traumatic thigh amputation (after a railway accident) before the accident. Assay of the coagulation status was only carried out as a consequence of a severe haemorrhage, results of which proved the presence of a mild haemophilia A with a Factor VIII activity of 9 %. Three packs of KRYOBULIN 500 were given daily to the patient over a period of two weeks. The patient was an adult with a body weight of 72 kilogrammes.

Daily control of the Factor VIII activity revealed values between 37 and 18 %. Bleeding was brought rapidly to a standstill. The considerable fluctuations of Factor VIII activity which occurred in this patient resulted from intermittent but not continuous transfusion.

In the case of the patient with amputation of one finger, haemophilia was also unknown before the operation. Only after a postoperative haemorrhage of one week's duration, coagulation assays were carried out and a mild haemophilia A with a Factor VIII activity of 6 % detected. After daily transfusions of three packs of KRYOBULIN 500, Factor VIII activity values between 30 and 36 % resulted and the haemorrhage stopped. The therapy was carried out over a period of two weeks

A rapid stop of haemorrhage was also seen in the cases of cerebral bleedings as well as with less dramatic bleedings such as haemarthroses, haematurias, haematomas and bone fractures. Substitution therapy with KRYOBULIN is also generally carried out in cases of dental extractions trying to maintain a 20 % Factor VIII level over a period of one week.

Dosage was ascertained for each patient and was calculated according to the Factor VIII activity desired. Only with haemarthroses with already well known patients who were mainly children between three and twelve years, 250 to 750 units of Factor VIII were administered within the first 24 hours.

Ordinarily, an assay of the PTT was carried out before treatment and at 12 to 24-hourly intervals after treatment.

Among the patients treated during the above mentioned time, one patient received 115 packs of KRYOBULIN 500, another 58 packs, two patients 27 pack each. The rest of the patients under test received between 23 and 15 packs. The lowest dose ever applied so far was a total of 6 packs of KRYOBULIN 250 in a 1968 born boy given for four incidents of haemarthroses.

3.5 Side Reactions

None of the above described cases developed hypervolemia as a consequence of massive treatment. The maximum volume of liquid administered was, however, 700 ml within a period or 24 hours in an adult.

In view of the fact that there was no case of an inhibitor developing against Factor VIII, there was no need for even more massive treatment with the resulting danger of hypervolemia.

The following side reactions occurred:

Chill with temperature over 39°C	1
Anaphylactic reactions	0
Haemolyses	0
Thrombocytopenia, consumption coagulopathy	0
Hepatitis	0

For detection of a possible latent hepatitis, 14 patients were observed at two to four week intervals over a period of 6 months following treatment. Attention was paid to SGPT, SGOT, LDH, bilirubin in the serum and urobilinogen in the urine. In no case was evidence of an overt or latent hepatitis found.

Linz, 20th November 1972

Helmut Vinazzer, M.D.

Specialist for Internal Medicine,
University Lecturer for Haematology
Linz, Austria

Krankenhaus der Stadt Wien-Lainz
Zentrallaboratorium
Vorstand: Prim. Univ.-Doz. Dr. med. M. Fischer
13 Wolkersbergenstraße 1, Telefon 82 26 11
1130 Wien

EINGEGANGEN
21. Jan. 1971

An die
Firma IMMUNO
Österr.Inst.f.Haemoderivate

Industriestraße 72
1220 Wien

Wien, am 19. Jänner 1971

Betr.: Anwendung der Plasmafraktion AHG_{500}, AHG_{250} bzw. Kryobulin

Seit dem Jahre 1965 wurde an der I. Medizinischen Universitätsklinik und verschiedenen Spitälern der Gemeinde Wien Kryobulin (AHG_{500}, AHG_{250}) zur Therapie der Hämophilie A mit Erfolg angewendet. Diese Plasmafraktionen (Kryobulin - AHG_{500}, AHG_{250}) waren Erzeugnisse der Fa. IMMUNO-AG, Österreichisches Institut für Haemoderivate, Wien 22, Industriestraße 72, Österreich.

Als Referenzen für die klinische Anwendung gebe ich folgende eigene wissenschaftliche Arbeiten an:

1) Fischer M., A.Zängl, P.H.Clodi, H.Karobath und K.Lechner; Magenresektion (B.II) bei Hämophilie A (Faktor-VIII-Mangel), Zentralblatt für Chirurgie, 91, 1805-1807, 1966

2) Fischer M. und A.Zängl; Zur Therapie gastrointestinaler Blutungen bei Hämophilie, Proc.Europ.Soc.Surg.1967, S. 656 Ceuterik, Leuvian, 1967

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4) Fischer M. und E.Deutsch; Therapie der Hämophilien, Deutsches Med.Journal, 19, 499-505, 1968

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Hochachtungsvoll

GRO-C

Prim.Univ.-Doz. Dr. med. M. Fischer
Vorstand des Zentrallaboratoriums

Translation

Krankenhaus der Stadt Wien - Lainz
Central Laboratory
Head: Prim.Univ.Doz.Dr.med.M.Fischer
13 Wolkersbergenstrasse 1, tel. 822611
1130 Vienna

Messrs.
IMMUNO
Österr. Inst.f. Haemoderivate
Industriestrasse 72
1220 Vienna

Vienna, January 19, 1971

Re: Application of Plasma Fraction AHG_{500}, AHG_{250} resp. Kryobulin

Since 1965 Kryobulin (AHG_{500}, AHG_{250}) has been successfully administered for treatment of Haemophilia A on the I. Medical University Clinic and several other hospitals in Vienna. These plasma fractions (Kryobulin - AHG_{500}, AHG_{250}) were produced by Messrs. IMMUNO AG, Österreichisches Institut für Haemoderivate, Vienna 22, Industriestrasse 72, Austria.

As references for the clinical application I give the following scientific publications of my own:

1) Fischer M., A. Zängl, P.H. Clodi, H. Karobath and K. Lechner: Magenresektion (B.II) bei Hämophilie A (Faktor-VIII-Mangel), Zentralblatt für Chirurgie, 91, 1805-1807, 1966.

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Yours sincerely,

sigd.

Prim.Univ.Doz. Dr. M. Fischer
Head of Central Laboratory

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Aus der I. Medizinischen Universitätsklinik Wien
(Vorstand: Prof. Dr. E. Deutsch)
und der II. Chirurgischen Universitätsklinik Wien
(Vorstand: Prof. Dr. H. Kunz)

Magenresektion (B. II) bei Hämophilie A (Faktor-VIII-Mangel)[1]

Von M. Fischer, A. Zängl, P. H. Clodi, H. Karobath und K. Lechner

[1] Vorgetragen in der wissenschaftlichen Sitzung der Gesellschaft der Ärzte in Wien, am 14. 1. 1966.

Vor etwas mehr als 10 Jahren endeten großchirurgische Eingriffe bei Patienten mit Hämophilie in der überwiegenden Mehrheit letal, und nur in wenigen Fällen, meist bei Patienten mit einem leichten Faktor-VIII-Mangel, konnte im Rahmen einer massiven Transfusionstherapie mit Frischblut das Leben erhalten werden.

Erst die Entwicklung hochkonzentrierter und hochgereinigter Präparate tierischen und menschlichen „Antihämophilen Globulins" zur Substitution des Faktor-VIII-Mangels ergab die Möglichkeit, bei Vorliegen einer Hämophilie A auch größere chirurgische Eingriffe mit Aussicht auf Erfolg wagen zu dürfen. Trotz dieser therapeutischen Fortschritte und verbesserter chirurgischer Technik ist aber die Indikation zu einer Operation nach wie vor äußerst streng und gewissenhaft zu stellen.

Gerade beim Vorliegen einer intestinalen Blutung bei einem Patienten mit einer Hämophilie, und zwar unabhängig von der Schwere der Erkrankung, ist die Gefahr der Verblutung sehr groß. Die konservative Therapie, selbst bei intensiver Substitution von Faktor VIII, ist nicht immer erfolgversprechend, so daß bei einem erwiesenen Ulcus ventriculi oder duodeni aus diesem Grunde und auch im Hinblick auf Rezidivblutungen die Indikation zur Operation heutzutage angezeigt erscheint.

An Hand der Schilderung des folgenden Falles wollen wir erläutern, daß nur intensives Zusammenwirken von Operateur und Internisten als Gerinnungsfachmann in allen Phasen der Vorbereitung, Durchführung und Nachbehandlung in einer derartigen Situation zielführend sein kann.

Bei einem nun 56jährigen Arzt (K.G.: Arch. Nr.: 101 544), dessen Familienanamnese unauffällig ist, kam es zum ersten Mal 1941 im Anschluß an eine Tonsillektomie zu einer lebensbedrohlichen Blutung, welche nur im Rahmen von wiederholten Bluttransfusionen beherrscht werden konnte. Schon damals wurde an das Vorliegen einer Hämophilie gedacht, aber keine Konsequenz daraus gezogen. 1948 kam es im Gefolge einer Nasenseptumoperation zu einer zweiten lebensbedrohlichen Blutung. 1960 führte zum ersten Mal eine schwere Melaena zur stationären Aufnahme und Behandlung in der II. Medizinischen Universitätsklinik. Auf Grund des Ergebnisses der im zentralen Gerinnungslaboratorium der I. Medizinischen Universitätsklinik durchgeführten Untersuchungen wurde nun erst die Diagnose einer Hämophilie A gestellt. In den Jahren 1961, 1963, 1964 erforderten rezidivierende schwere gastrointestinale Blutungen stationäre Behandlungen an der I. Medizinischen Universitätsklinik. Wiederholte Frischbluttransfusionen, hohe Dosen von antihämophilem Plasma und Cohn-Fraktion I brachten die Blutung jeweils zum Stillstand. Klinik und Verlauf (Sodbrennen, Nüchternschmerz und Druckschmerz rechts vom Nabel und langdauernde Hyperaziditätsbeschwerden) ließen als Blutungsursache ein Ulcus duodeni als wahrscheinlich annehmen. 1963 konnte auch röntgenologisch ein Ulcus duodeni nachgewiesen werden. Bis zur letzten Aufnahme 1964 gab der Patient keine Einwilligung zu einer Operation.

Am 8. 2. 1965 erfolgte die neuerliche Aufnahme in der I. Medizinischen Universitätsklinik wegen schwerer Melaena und Kollaps. Mit der üblichen Therapie — Transfusionen, Plasmainfusionen, Cohn-Fraktion I — konnte diesmal die Blutung nicht beherrscht werden. Nach reiflichster Überlegung und gemeinsamer Planung stellten wir am 12. 2. 1965 in einem nächtlichen Konsilium die Anzeige zur chirurgischen Intervention.

Die Bauchhöhle wurde durch eine schräge Laparotomie eröffnet, wobei sofort der prall mit Blut gefüllte Darm auffiel. Bei äußerer Inspektion war ein Ulkus zunächst nicht auffindbar. Nach präpylorischer Gastrotomie konnte durch Einsetzen von Häkchen das Innere des Duodenums inspiziert werden. Es fand sich ein ins Pankreas penetrierendes, bohnengroßes blutendes Ulkus. Wir führten die typische ⅔-Resektion mit antekolischer Anastomose aus. Um einen Rückstau in den Duodenalstumpf zu verhüten, fügten wir eine Braunsche Enteroanastomose hinzu. Der Eingriff verlief in allen Phasen typisch, wir befleißigten uns einer möglichst atraumatischen Technik mit Verzicht auf Verwendung des elektrischen Messers. Alle erkennbaren Blutpunkte wurden mit feinen Durchstechungsligaturen versorgt. Mit Ausnahme einer subkutanen Lasche, welche nach 24 Stunden entfernt wurde, erfolgte der Wundschluß drainagelos.

Der postoperative Verlauf war völlig komplikationslos. Am 5. Tag nach dem Eingriff konnten wir den Patienten zur weiteren Betreuung in die I. Medizinische Universitäts-Klinik rücktransferieren, da ja der Akzent der weiteren Therapie in der Gewährleistung der Hämostase lag. Die Wundheilung erfolgte per primam, die Nahtentfernung am 12. postoperativen Tag.

Der Patient fühlt sich weitgehend beschwerdefrei und ist seit einem Jahr in seiner ausgedehnten ärztlichen Praxis voll einsatzfähig.

Tabelle I

		Normalwert
Gerinnungszeit	18′	– 9′
Blutungszeit	1′45″	–3′
Thrombozyten	220000	150–400000
Thromboplastinzeit	100%	75–110%
Faktor II	100%	75–110%
Faktor V	100%	75–110%
Faktor VII	100%	75–110%
Faktor X	90%	75–110%
Fibrinogen	260 mg%	200–400 mg%
Partielle Thromboplastinzeit	119 sec	55–70 sec
Thromboplastin Bildungstest	Faktor-VIII-Mangel	
Quant. Faktor VIII	34%	70–200%
Serum-Prothrombin nach 1 h	60%	weniger als 10%

Im Tauchversuch konnte kein Hemmkörper nachgewiesen werden.

An Hand dieser Befunde konnte die Diagnose einer Subhämophilie A gestellt werden.

Zu Beginn des Jahres 1965 stand uns erstmals eine neue Plasmafraktion mit einer hohen Konzentration an menschlichem Faktor VIII zur Verfügung. Es handelt sich um das Präparat AHG_{500} und AHG_{250} der Fa. Immuno-Wien. Der Inhalt einer Packung, in 100 ml pyrogenfreiem Wasser aufgelöst, entspricht einer Faktor-VIII-Aktivität von 500 ml bzw. 250 ml Frischplasma. Damit hat man den Vorteil, dem Patienten in einer kleinen Flüssigkeitsmenge eine hohe Konzentration an Faktor VIII zuzuführen. In Anbetracht der leichten Hämophilie A bei diesem Patienten haben wir uns zu diesem Präparat entschlossen.

Unmittelbar vor der Operation erhielt der Patient 3 E AHG_{500} und 1 E AHG_{250}, das entspricht einem Äquivalent an Faktor VIII von 1750 ml Frischplasma. Damit konnte ein Faktor-VIII-Spiegel von 107% im Plasma des Patienten erreicht werden. Die erhöhte Blutungsneigung war nun von seiten der hämorrhagischen Diathese ausgeschaltet, und der operative Eingriff konnte begonnen werden. Im weiteren Verlauf der Operation wurden noch 4 E AHG_{250} – ein Äquivalent von 1000 ml Frischplasma – verabreicht, der Faktor-VIII-Spiegel stieg auf 160% an.

Postoperativ wurden insgesamt 14 E AHG_{500}, 24 E AHG_{250} und 75 E Cohn-Fraktion I dem Patienten verabreicht. Das entspräche einem Frischplasma-Äquivalent von rund 28 l. Die Einzeldosen wurden bis zum 9. postoperativen Tag 4stündlich und bis zum 12. postoperativen Tag 6stündlich verabreicht. Während und nach der Operation kam es zu keiner Blutung, die Wunde heilte per primam. Am zweiten postoperativen Tag kam es im Bereich der Vene, in welcher der Dauerkatheter lag, zu einer Thrombose. Diese Beobachtung kann man ihm Rahmen einer hochdosierten Substitutionstherapie mit menschlichen und tierischen AGH-Präparaten wiederholt feststellen.

Trotz dieser geglückten Operation möchten wir aber nochmals mit Nachdruck darauf hinweisen, daß chirurgische Eingriffe bei hämorrhagischen Diathesen auch heute, wo menschliche und tierische hochkonzentrierte Plasmafraktionen als Therapie zur Verfügung stehen, noch immer ein erhöhtes Risiko haben. Dabei sei nur an

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erinnert.
Der Aufw
einer guten Z
Maß reduziert

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Bachmann, W.
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Fenninger und E.
digl, Amer. J. Pedi
Eldin, M. G., und
Wien. klin. Wschr.
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das plötzliche Auftreten eines Hemmkörpers während der Substitutionstherapie erinnert.

Der Aufwand solcher Operationen erscheint groß, doch läßt er sich im Rahmen einer guten Zusammenarbeit von Chirurgen und Internisten auf ein vernünftiges Maß reduzieren.

Zusammenfassung

Bei einem 56jährigen Patienten mit einer Subhämophilie A (Faktor VIII — 34%) bestand wegen rezidivierender Ulcera duodeni die Indikation zu einer Magenresektion nach Billroth II. Im Rahmen der prä-, per- und postoperativen Substitution von Faktor VIII mit menschlicher Cohn-Fraktion I verlief der chirurgische Eingriff und die Wundheilung komplikationslos.

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Anschr. d. Verf.: Dr. M. Fischer, I. Medizinische Universitätsklinik, 1095 Wien 9, Spitalgasse 23, Doz. Dr. A. Zängl, II. Chirurgische Universitätsklinik, 1095 Wien 9, Spitalgasse 23

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Billroth's Operation (B.II) in Cases of Hemophilia A (Factor VIII Deficiency)

M.Fischer, A.Züngl, P.H.Clodi, H.Karobath and K.Lechner

At the beginning of 1965 we first had a new plasma fraction with a high concentration in human factor VIII at our disposal, the preparation ANTIHAEMOPHILIC GLOBULIN "500" and ANTIHAEMOPHILIC GLOBULIN "250" of the firm of Immuno-Vienna. The content of one bottle - diluted in 100 ml pyrogenfree water - corresponds to a factor VIII activity of 500 ml or 250 ml fresh plasma. This way the patient can receive a high concentration in factor VIII in a small amount of liquid. We decided to use this preparation in a patient with hemophilia A who had to undergo abdominal surgery.

Prior to the operation the patient received 3 units AHG "500" and 1 unit AHG "250" which corresponds to a factor VIII content of 1750 ml fresh plasma. A factor VIII level of 107 % in the plasma of the patient was achieved. Thus increased tendency to bleeding was eliminated and the operation could be started. During the operation an additional 4 units AHG "250" equivalent to 1000 ml fresh plasma were administered, the factor VIII level increased to 160 %.

Post-operatively a total of 14 units AHG "500", 24 units AHG "250" and 75 units Plasma Fraction I (Cohn) were given. This corresponds to an equivalent of approx. 25 l fresh plasma. The single doses were administered until the 9th post-operative day at intervals of 4 hours, until the 12th post-operative day at intervals of 6 hours. During and after the operation no bleeding occurred the wound healed per primam. On the 2nd post-operative day a thrombosis occurred in the area of the vene with the permanent catheter. High doses of human and animal AHG preparation often led to local thrombosis.

..../2

Summing up, it can be said that a 56 year old patient suffering from sub-hemophilia A (factor VIII - 34 %) had to undergo a Billroth II operation because of recurrent ulcera duodeni. Owing to the pre-, per- and post operative substitution of factor VIII with human Plasma Fraction I (Cohn) surgery and healing of the wound were without complications.

Proc. Congr. Soc. Int. Chir. 1967 in Wien, Centaurick, Louvain, 1968

ZUR THERAPIE GASTROINTESTINALER BLUTUNGEN BEI HAEMOPHILIE

M. FISCHER* und A. ZÄNGL (**) (Wien)

Gastrointestinale Blutungen sind bei der Hämophilie nicht ungewöhnlich.

Die Angaben in der Literatur (Achenbach[1], Deutsch[4], Ikkala[7], Kerr[8], Ramgren[10] u.a.) bezüglich der Häufigkeit dieser Blutungen sind allerdings recht unterschiedlich und schwanken zwischen 10-42% der Fälle. Gastrointestinale Blutungen, die selbst beim blutgerinnungsnormalen Patienten zu schweren, lebensbedrohlichen Zuständen führen können, stellen naturgemäß bei Patienten mit einer Blutgerinnungsstörung, wie der Haemophilie, ein wesentlich größeres Risiko dar; die Gefahr der Verblutung ist beträchtlich. Trotzdem wird man sich zunächst in einer solchen Situation konservativ verhalten, d.h. das therapeutische Ziel ist primär die Sanierung der Gerinnungsstörung. Bei Vorliegen einer Haemophilie A (Faktor VIII-Mangel) müssen ausreichende Mengen Faktor VIII-hältiger Plasmafraktionen verabreicht werden. Handelt es sich um die seltene Haemophilie B (Faktor IX-Mangel), so soll die Behandlung mit Faktor IX-hältigen Plasmafraktionen erfolgen. Die Substitutionstherapie muß unabhänging von der Schwere der Haemophilie (Tab. I) konsequent und intensiv durchgeführt werden, wobei der Faktor VIII oder IX-Spiegel auf 30-40% gebracht werden soll.

Tabelle I

BEURTEILUNG DES SCHWEREGRADES DER HÄMOPHILIE A UND DER HÄMOPHILIE B

Faktor VIII- oder IX-spiegel im Plasma des Patienten	Schweregrad der Erkrankung
bis 1%	schwer
1-5%	mittelschwer
5-15%	leicht
über 15%	Subhämophilie
75-200%	normal

(*) I. Medizinische Universitätsklinik.
(**) II. Chirurgische Universitätsklinik

In der Tabelle II sind die in Verwendung stehenden Plasmafraktionen sowie ein allgemeines Dosierungsschema angeführt; ansich erfordert aber jeder Patient eine individuelle Behandlung.

Tabelle II

PLASMAFRAKTIONEN, WELCHE FÜR DIE BEHANDLUNG DER HÄMOPHILIE A VERWENDET WERDEN:

Name der Präparation	Faktor VIII-Gehalt	Der Inhalt einer Flasche entspricht einem Plasmaäquivalent von
Plasmafraktion I n.COHN	100 - 250%	100 ml
Menschliches Antihämophiles Globulin „250"	176 - 328%	250 ml
Menschliches Antihämophiles Globulin „500"	170 - 400%	500 ml
Plasmafraktion 1-0 n. Blombäck	320 - 450%	500 ml
Antihämophiles Globulin von Tier (Schwein, Rind)	800 - 1169%	800 ml

Präparate, welche für die Behandlung der Hämophilie B verwendet werden:

Name der Präparation	Faktor IX-Gehalt	Der Inhalt einer Flasche entspricht einem Plasmaäquivalent von
Antihämophiles Plasma (Frischgefrorenes, lyophilisiertes Plasma)	80 - 200%	100 ml
„P.P.S.B."	150 - 300%	250 ml

Die Dosierung dieser Präparate hat so zu erfolgen, daß bei dem Patienten der Faktor VIII- oder IX-Spiegel im Blut vor, während und nach der Operation auf über 50% angestiegen ist. Hierfür sind 10-40 ml Plasma/kg Körpergewicht 4-8 mal täglich erforderlich, was 3-5 L Plasma u.m. pro Tag entspricht. Zunächst wird man versuchen, dies durch Gabe von menschlichen Präparaten zu erreichen, doch erweist es sich manchmal als unumgänglich auch tierische Präparationen zu geben. Bei diesen kommt es aber nach 8-10 Tagen zu einer Sensibilisierung gegenüber dem tierischen Eiweiß, d.h. das Plasma einer Spezies ist nur für eine Periode von 8-10 Tagen verabreichbar.

Noch vor mehr als 10 Jahren, als die Therapie der Haemophilie zum Großteil mit Frischblut, Frischplasma und Konservenblut durchgeführt wurde und daher die Substitution von Faktor VIII bzw. Faktor IX zumeist nur unzureichend war, betrug die Letalität der gastrointestinalen Blutung

ca 50% (Ramgren[10].) Mit der Entwicklung besonderer an Faktor VIII — bzw. Faktor IX angereicherter Plasmafraktionen verbesserte sich die Situation wesentlich, trotzdem betrug die Letalität nach Ikkala[7] immer noch 9%.

Von den 220 Patienten mit Haemophilie A und B, welche wir seit 1950 an der I. Med. Univ. Klinik in Wien diagnostizierten und zum Teil behandeln konnten, hatten 21 gastrointestinale Blutungen; 9 dieser Patienten wurden vorwiegend mit Blutungen aus einem Ulcus ventriculi bzw. duodeni an der Klinik stationär aufgenommen (Tab. III). Im Rahmen einer intensiven Substitution mit AHP und Plasmafraktion I n. Cohn war die konservative Behandlung in 8 Fällen, welche zum Teil auch rezidivierende Blutungen aus einem Ulcus ventriculi oder duodeni hatten, immer erfolgreich. Als Beispiel sei der Verlauf bei einem 39 Jahr alten Mann mit einer Hämophilie A (Faktor VIII, 3,9%) angeführt. (KG Nr. 189/67).

Diesem primär konservativen Vorgehen sind aber auch eindeutige Grenzen gesetzt, sodaß trotz aller Zurückhaltung gegenüber einem chirurgischen Vorgehen bei hämophilien Patienten die Operation gelegentlich

Tabelle III

HÄUFIGKEIT DER GASTRO-INTESTINALEN BLUTUNGEN BEI PATIENTEN MIT HÄMOPHILIE

Schweregrade der Hämophilie	Patienten mit gastro-intestinale Blutungen		Operationen
	einmalig	recidivierend	
schwer	10	3	—
mittelschwer	2	1	—
leicht	3	1	1(*)
Subhämophilie	—	1	1

(*) Dieser Patient wurde in einem anderen Spital operiert.

FA: Ein Bruder ist auch Bluter. Schon seit der Kindheit hat der Patient nach größeren Traumen eine besondere Neigung zu Haematomen. 1935 kam es nach einer Tonsillektomie zu einer schwerden Nachblutung. 1960 wurde die Haemophilie A (Faktor VIII 3,9%) bestätigt. Im Januar 1967, nachdem ca 9 Monate vorher zunehmende Schmerzen im Oberbauch (Nüchternschmerz) und einer Hyperacidität bestanden, kam es zu einer schweren Hämatemesis und Melaena; der Patient wurde in ein Spital eingewiesen. Auf Transfusionen von Konservenblut und auch Frischblut (insgesamt ca 1500 ml Blut) kam es zu keiner Besserung, das Blutbild verschlechterte sich (HK 10%) zusehens. Daraufhin erfolgte die Transferierung an die I. Med. Univ. Klinik. Es wurde sofort mit einer intensiven Therapie mit AHP 500 Cohnfraktion I und Frischblut begonnen. In den ersten 3 Tagen erhielt der Patient 9 E AHG 5000 und 34 E Cohnfraktion I, das entspricht ca einen Äquivalent von 12 000 ml Plasma. Die Blutung war im Rahmen dieser Therapie schon am 2. Tag zum Stillstand gekommen. Die Röntgenuntersuchung von Magen und Duodenum am Ende des Klinikaufenthaltes ergab das Vorliegen des abgeheilten Ulcus duodeni. Der Patient konnte beschwerdefrei entlassen werden.

doch als vital indiziert durchgeführt werden muß:

1) Wenn die gastrointestinale Blutung trotz weitgehender Normalisierung der Gerinnungsstörung durch Gabe menschlicher, hochkonzentrierter Faktor VIII-. oder IX. Präparationen unvermindert andauert und lebensbedrochliche Ausmasse annimmt, sodass der Einsatz konzentrierter tierischer Präparate erwogen wird. Dabei mag es gleichgültig sein, ob als Blutungsursache ein Ulcus ventriculi oder duodeni nachweisbar war oder nicht. Diese Situation gleicht jener, bei welcher auch beim blutgerinnungsnormalen Patienten mittels sogenannter „Resektion im Blinden" die Blutung beherrscht und das Leben des Patienten gerettet werden kann. Allerdings muß man bei der Gabe dieser therapeutisch sehr wirksamen tierischen Präparate wissen, daß man Präparationen einer Species nur ca. 8-10 Tage anwenden kann, dann kommt es zur Sensibilisierung gegen das tierische Protein. Mitunter sind auch akute Verminderungen der Thrombozytenzahl während dieser Behandlung beschrieben worden. (Loeliger[9], Biggs[3]).

2) Wenn es bei nachgewiesenem Ulcus ventriculi bzw. duodeni trotz intensiver interner Behandlung — medikamentös, diätetisch — in kurzer Zeit mehrmals zu schweren lebensbedrohlichen Blutungsepisoden kam, sodaß die Operation eine vitale Indikation darstellt.

3) Bei evt. Vorliegen einer freien Perforation, da die Größe der Perforationsöffnung nicht bestimmbar ist und der Versuch, diese Komplikation durch Absaugen zu beherrschen, sehr problematisch bleibt. Trotz dieser allgemeinen Richtlinien muß nochmals vor einem schematischen Vorgehen gewarnt werden. Die Situation bei jedem einzelnen Patienten muß in intensivem Zusammenwirken von Chirurgen und Internisten verantwortungsvoll und exakt besprochen werden. Als Beispiel sei ein Patient angeführt, bei welchem die Therapie mit menschlichen Plasma-Fraktionen zur Beherrschung der Blutung nicht gelang und eine Operation vital indiziert war.

B. R., männlich, geb. 1909 (KGNr: 101 544): FA unauffällig, 1941 im Anschluß an eine Tonsillektomie kam es zu einer lebensbedrohlichen Blutung. 1948 trat eine schwere Blutung nach einer Nasenseptumoperation auf. 1960 wurde im Anschluß an eine schwere Melaena bei einem Ulcus duodeni im Rahmen einer Gerinnungsuntersuchung erstmals eine Hämophilie A (Faktor VIII 34%) festgestellt. 1961, 1963, 1964 rezidivierende schwere gastrointestinale Blutungen, die aber jeweils mit hohen Dosen von Antihämophilem Plasma sowie Cohn-Fraktion I beherrscht werden konnten. Eine schwere, mit diesen Plasmafraktionen nicht mehr zu stillende Ulcusblutung Anfang 1965 war die vitale Indikation zur Magenresektion (B II). Aus der Tab. V ist der Therapieverlauf zu ersehen. Präoperativ erhielt der Patient in Form von AHG 500 bzw. AHG 250 ein Äquivalent von 1750 ml Frischplasma. Damit konnte ein Faktor VIII-Spiegel von 107% erreicht werden. Während der Operation wurde ein Äquivalent von 1000 ml Frischplasma gegeben, der Faktor VIII stieg auf 160%. In der postop. Phase wurden ingesamt 14 E AHG, 500, 24 E AHG 250 und 75 E Cohn-Fraktion I verabreicht, was zusammen einem Äquivalent von 28 000 ml Frischplasma entspricht. Die Applikation von AHG bzw. Cohn-Fraktion I erfolgte in den ersten 9 Tagen 4-stündlich, dann 6-stündlich. Der postop. Verlauf war ohne Blutungskomplikationen, die Wunden heilten p.p. Lediglich am 2. postop. Tage kam es im Bereiche der Vene, in welcher der Dauerkatheter eingeführt war, zu einer Thrombose. Der Patient steht weiterhin in ambulanter Kontrolle und ist völlig beschwerdefrei. Eine gastrointestinale Blutung ereignete sich nicht mehr.

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ZUSAMMENFASSUNG

Bei Patienten mit Hämophilie und gastrointestinale Blutungen ist die konservative Therapie, selbst bei intensiver Substitution vom Faktor VIII bzw. IX, nicht immer erfolgversprechend, so daß mitunter die Indikation zur Operation vital angezeigt erscheint. Solche chirurgische Eingriffe unter der Substitutionstherapie mit menschlichen und tierischen hochkonzentrierten Plasmafraktionen stellen zwar auch ein erhöhtes Risiko dar, es sei nur an das plötzliche Auftreten eines Hemmkörpers erinnert, doch wurden schon eine Reihe geglückter Operationen mitgeteilt.

Der Aufwand solcher Operationen erscheint groß, doch wird er von therapeutischem Erfolg gerechtfertigt.

*

In haemophilic patients with gastro-intestinal haemorrhage conservative treatment is not invariably successful. Even where there is a high level of Factor VIII and IX, operation may be urgently indicated.

This is not however without some risk because if concentrated human or animal plasma is given they may produce a sudden antibody reaction. In spite of this risk a good many successful cases have been recorded.

*

En los pacientes con hemofilia y hemorragia gastrointestinal, la terapéutica conservadora, incluso cuando se hace una substitución intensiva del factor VIII o IX no siempre proporciona los resultados deseados. Por lo cual, se plantea la indicación vital de intervenir. Tal intervención bajo los

efectos de una terapéutica substitutiva con fracciones de plasma animal o humano a altas concentraciones significan, por su parte, un elevado riesgo, aunque sólo sea por la irrupción brusca de substancias inhibidoras. A pesar de lo cual han sido comunicados una serie de casos de buen resultado operatorio.

Se trata de una operación de magnitud, pero que parece justificada por los resultados terapéuticos.

*

Chez les hémophiles atteints d'hémorragie digestive, les traitements conservateurs peuvent échouer, même si l'on réalise une thérapeutique intensive par les facteurs VIII et IX. L'indication opératoire paraît donc impérieuse. L'intervention chirurgicale présente évidemment un risque certain, même lorsqu'on la combine aux thérapeutiques de substitution avec des fractions plasmatiques très concentrées. Mais plusieurs interventions réussies prouvent que cette attitude est justifiée.

*

Negli emofiliaci colpiti da emorragia digestiva, le cure conservatrici possono fallire, anche se si realizza una cura intensiva con i fattori VIII e IX. L'indicaziono operatoria è dunque imperativa. L'intervento chirurgico presenta evidentemente un rischio sicuro, anche quando si associ alla terapia sostitutiva una terapia di frazioni plasmatiche molto concentrate. Molti interventi riusciti provano che questa condotta è giustificata.

*

У пациентов с гемофилией и желудочно-кишечными кровотечениями консервативная терапия сама по себе при интенсивной субституции фактора VIII или IX не всегда приносит успех, так что нам кажется, что иногда показания к операции абсолютны. Такие хирургические вмешательства при субституционной терапии высококонцентрированной фракцией плазмы человека или животных представляют собой большой риск (следует вспомнить о внезапном появлении тормозящего тела).

Сообщается о ряде успешных операций.

Therapy of Gastrointestinal Bleedings in Cases of Hemophilia

M.Fischer and A.Zängl (Vienna)

B.R., male, born in 1909: family history uneventful, in 1941 following tonsillectomy a dangerous bleeding and in 1948 serious bleeding following a nasal septum operation occurred. 1960 following melena ulcus duodeny was diagnosed. Hemophilia A (factor VIII 34 %) first was discovered on this occasion. 1961, 1963, 1964 recurrent serious gastrointestinal bleedings occurred which, however, could be kept under control with high doses of Antihaemophilic Plasma and Cohn Fraction I. At the beginning of 1965 a serious ulcer bleeding not to be controlled anymore by these plasma fractions was the vital indication for a Billroth's operation (B II). For therapy schedule see fig. 1.

Before operation the patient received an equivalent of 1750 ml fresh plasma in form of AHG "500" or AHG "250". A factor VIII level of 107 % was achieved. During the operation an equivalent of 1000 ml fresh plasma was administered; factor VIII increased to 160 %. In the post-operative phase a total of 14 units AHG "500", 24 units AHG "250" and 75 units Cohn Fraction I were given, amounting to an equivalent of 28,000 ml fresh plasma. During the first 9 days AHG and/or Cohn Fraction I were administered at intervals of 4 hours, then at intervals of 6 hours. After the operation no bleeding complications occurred, the wound healed p.p. Only on the 2nd post-operative day thrombosis occurred within the area of the venous catheter. The patient is still under control as an outpatient and entirely free of symptoms. A gastro-intestinal bleeding has not occurred since.