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PRODUCT LICENCE APPLICATION

FACTORATE

VOLUME II

CLINICAL STUDIES

AP000915

ACCO

ARMOUR000123

CONFIDENTIAL
COPY No....

MEDICINES ACT 1968

APPLICATION FOR A PRODUCT LICENCE

FACTORATE

VOLUME II - CLINICAL STUDIES

INDEX

SGB/FK
March, 1975
Armour Pharmaceutical Co. Ltd.,
Hampden Park,
Eastbourne,
Sussex.

MEDICINES ACTS 1968 AND 1971 - APPLICATION FOR PRODUCT LICENCE

Full name and address of proposed licence holder: Armour Pharmaceutical Co. Ltd.,
Hampden Park,
Eastbourne, Sussex

Trading style to be shown on licence if different from above: N/A

Role of proposed licence holder:
(i) ~~as person responsible for organisation of product's manufacture and/or importation~~
(ii) as person who imports or procures its importation
(iii) as person who first sells or supplies it as a medicinal product

Activities for which licence is required:
(i) selling or supplying product in the UK
(ii) procuring the manufacture or assembly of the product for sale or supply in the UK
(iii) importing or procuring the importation of the product
(iv)

Applicants own reference no: SGB/FK

Name of Product: FACTORATE (FACTOR VIII)

Fees information:
(i) Does this product attract the major initial fee? No ~~Yes/No~~
(ii) If so, do you wish to pay this fee by instalments? ~~Yes/No~~
(iii) Is this product exempt from the initial fee? Yes ~~Yes/No~~
(iv) Do you hold any other product licence for which you are paying a duration fee? ~~Yes~~/No
(v) Are you a retail pharmacist? ~~Yes~~/No
(vi) Do you hold a manufacturer's licence? Yes~~/No~~
(v) Are you a retail pharmacist? ~~Yes~~/No
(vi) Do you hold a manufacturer's licence? Yes/~~No~~
(vii) Will this product be manufactured by a licensed manufacturer? ~~Yes/No~~ Yes
(viii) Do you wish any surcharge to be adjusted by reference to sales? ~~Yes~~/No

~~I~~/We apply for the grant of a product licence to the proposed holder named above in respect of the product(s) to which the Product Particulars on page 2 refer and in accordance with the other particulars annexed; the said licence to be for a period of five years and subject to the following provisions:-

1. All the provisions of Part I of Schedule 1 of the Medicines (Standard Provisions for Licences and Certificates) Regulations 1971 (S.I. 1971 No 1972) shall apply.
2. The product shall not be recommended to be used for any purpose other than those specified in the Product Particulars as Uses.
3. The specifications of the constituents and of the finished product shall be in accordance with information contained in or furnished in connection with the application.
4. The product is to be manufactured only in accordance with the methods set out in this application or furnished in connection with it.
5. The number of the Licence shall appear on all containers or packages in which the product(s) is/~~are~~ packed and on any package inserts or accompanying literature.

Date 26th March 1975 Signature GRO-C
State capacity in which signed (Head of Regulatory Affairs)

Name and address for communications:-
Mr.S.G.Brooks
Armour Pharmaceutical Co. Ltd.,
Eastbourne, Sussex

Scientific Evidence:
(i) Chemistry and Pharmacy 104 pages
(ii) Experimental and Biological Studies – pages
(iii) Clinical Trials 52 pages

Number of pages of supplementary information:- 2

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For licensing authority use

Product Particulars

2.1 Name of Product: FACTORATE (FACTOR VIII)

2.2 Pharmaceutical form: Injection. The preparation is in a form for administration to human beings.

2.3 Active constituents: Antihaemophilic Factor (Human)

2.4 Uses: In therapy of classical haemophilia (Haemophilia A).

Route of administration - intravenous infusion and intravenous injection

2.5 Recommended dose and dosage schedules: Dosage must be individualised according to needs of the patient. Full recommended general dosages are given in the package insert leaflet.

2.6 Contra-indications: There are no known contraindications to anti-haemophilic factor.

2.7 Method of retail sale or supply: Supplied in single dose vials with the stated antihaemophilic factor activity on the label. Also supplied if required - a vial of diluent and sterile needles for reconstitution, withdrawal and injection.

2.8 Manufacturer of dosage forms: Armour Pharmaceutical Company, Phoenix, Arizona 85077 U.S.A.

Applicants reference number (as on page 1) SGB/FK

Applicants signature ... GRO-C .....

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SUPPLEMENTARY PARTICULARS

3.1. Physical Characteristics

A white to pale yellow lyophilised cake in a vial with vacuum.

3.2. Manufacture

(a) Factorate is manufactured from fresh frozen human plasma which when tested is found to be negative for hepatitis B antigen activity. Cryoprecipitate as collected from thawed human plasma is dissolved in a buffer containing glycine, sodium chloride, and heparin. Impurities such as prothrombin are adsorbed with aluminium hydroxide after which the preparation is stablized with sodium citrate and heparin in an isotonic medium. Following the addition of stabilizers the solution is sterile filtered, filled into vials, and lyophilised.

(b) The Quali-quantitative formula:-

Factor VIII (Lyophilised)	NLT 125 U/Vial
Glycine	0.02 Molar
Sodium Chloride	0.04 Molar
Sodium Citrate	0.04 Molar
Heparin	NMT $2\frac{1}{2}$ U/ml

(c) The Name & Address of Place of Manufacture Assembly

Armour Pharmaceutical Company,
P.O. Box 511,
Kankakee,
Illinois, 60901
U.S.A.

(d) The Names & Addresses of Manufacturers of the Active Constituents

As in (c) above, together with Metrix Clinical & Diagnostics Division, Armour Pharmaceutical Co., Chicago, Illinois. 60616.

(e) The Arrangements for Storage and place of such Storage

The product is stored at -40°C during quarantine prior to labelling. It is stored at 2 - 8°C after labelling and held at Armour Pharmaceutical Co. P.O. Box 511, Kankakee, Illinois 60901.

3.3 Quality Control

Quality Control will be exercised on the raw materials, the ingredients used in the process during pharmaceutical manufacture and on the finished product. The vial appearance and fill weight is checked, tests for sterility and safety are carried out in accordance with Public Health Service Regulations, Title 42, Part 73 paragraphs 73.720 and 73.730 respectively.

3.4 Containers

The vials are 50 ml USP Type I glass with 20 mm finish closed with grey butyl lyophilisation style stoppers and having on aluminium flip-off seal.

3.5 Labelling

As provided in Volume II.

APPENDIX TO SUPPLEMENTARY INFORMATION
TO VOLUME I & II

3.3 Quality Control (Cont.)

Whether the proposed licensee will be responsible for deciding if any batch of the product is of acceptable quality for marketing.

In accordance with the "Guide to Good Pharmaceutical Manufacturing Practice" the Quality Controller at Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, will be responsible for the control of quality of products made by Armour Pharmaceutical Co., Kankakee, Illinois.

Safety Tests Employed

The test is performed as described in Public Health Service Regulations, Title 42, Part 273, Paragraph 73.720.*

Dosage into mice : 0.5 ml
Dosage into Guinea Pigs : 5.0 ml

Bulk and Final Container Tests for Sterility

The inoculum for final containers after reconstruction :-

1 ml in fluid Thioglycollate Medium
1 ml in Soyabean Casein digest

The above regulations apply*

* Food and Drug Regulations are as attached.

of all processes of manufacture which may affect compliance with the standard to which the test applies. The results of all tests performed shall be considered in determining whether or not the test results meet the test objective, except that a test result may be disregarded when it is established that the test is invalid due to causes unrelated to the product.

§ 73.710 Potency.

Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 73.101(t).

§ 73.720 General safety.

In addition to specified safety tests prescribed in this part for individual products, a general safety test shall be performed in final container material, from each filling of each lot of all products intended for administration to man, either after the labels have been affixed to the final container, or affixed, both outside and inside, to the multiple container storage receptacle just prior to its sealing for storage purposes. Exceptions to this procedure may be authorized by the Director, Division of Biologics Standards, when more than one lot is processed each day. The general safety test shall consist of the parenteral injection of the maximum volume tolerated into each of two mice weighing approximately 20 gms. each and into each of two guinea pigs weighing approximately 350 gms. each but no more than 0.5 ml. need be inoculated into each mouse and no more than 5.0 ml. need be inoculated into each guinea pig. After injection the animals shall be observed for a period of no less than seven days and if neither significant symptoms nor death results during the observation period, the product meets the requirements for general safety. Variations of this test, either in the volume injected or in the species of test animal used shall be made whenever required because of the human dose level demanded of the product or because of any individual demands of the product itself.

§ 73.730 Sterility.

Except as provided in paragraph (f), the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material. Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels.

(a) *The test*—(1) *Using Fluid Thioglycollate Medium.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the "inoculum"), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30° to 32° C. for a test period of no less than seven days and examined visually for evidence of growth on the third or fourth or fifth day and on the seventh or eighth day. If incubation is continued beyond eight days, an additional examination shall be made on the last day of the test period. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 ml. shall be transferred on the third or fourth or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of medium. The material in the additional vessels shall be incubated at a temperature of 30° to 32° C. for no less than seven days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third or fourth or fifth day of incubation and on the seventh or eighth day and if incubation is continued beyond a period of eight days, an additional examination shall be made on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (e) of this section.

(2) *Using Fluid Sabouraud Medium.* Except

for dried products, a test for fungi and yeast shall be made on final container material, following the procedures prescribed in subparagraph (1) of this paragraph except that (i) the medium shall be Fluid Sabouraud Medium; (ii) the incubation shall be at a temperature of 20° to 25° C.; (iii) the period of incubation shall be no less than ten days and an examination shall be made on the tenth or eleventh or twelfth day in lieu of an examination on the seventh or eighth day.

(b) *Repeat tests*—(1) *Repeat bulk test.* If growth appears in the test of the bulk material, the test may be repeated to rule out faulty test procedures by testing at least the same volume of material.

(2) *First repeat final container test.* If growth appears in any test (thioglycollate or Sabouraud) of final container material, that test may be repeated to rule out faulty test procedures by testing material from a sample of at least the same number of final containers.

(3) *Second repeat final container test.* If growth appears in any first repeat final container test (thioglycollate or Sabouraud), that test may be repeated provided there was no evidence of growth in any test of the bulk material and material from a sample of twice the number of final containers used in the first test is tested by the same method used in the first test.

(c) *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b)(2) or (b)(3) of this section, whichever is applicable.

(d) *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (f) of this section.)

(2) *Final containers.* The sample for the final container and first repeat final container tests shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel. If the amount of material in the final container is 1.0 ml. or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 ml., the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 ml., whichever is larger, but no more than 10 ml. of material or the entire contents from a single final container need be tested. (Note exceptions in paragraph (f) of this section.)

(e) *Culture medium*—(1) *Formulae.* (i) The formula for Fluid Thioglycollate Medium is as follows:

Fluid Thioglycollate Medium

L-cystine	0.5 gm.
Sodium chloride	2.5 gm.
($C_6H_{12}O_6 \cdot H_2O$) Dextrose	5.5 gm.
Granular agar (less than 15% moisture by weight)	0.75 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest of casein	15.0 gm.
Purified water	1,000 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.)	0.5 gm.
Resazurin (0.10% solution, freshly prepared)	1.0 ml.

Final pH 7.1±0.1.

(ii) The formula for Fluid Sabouraud Medium is as follows:

Fluid Sabouraud Medium

Dextrose	20 gm.
Pancreatic digest of casein	5 gm.
Peptic digest of animal tissue	5 gm.
Purified water	1,000 ml.

Final pH 5.7±0.1.

(2) *Culture medium requirements*—(i) *Quality and condition of medium and design of test vessel.* The growth promoting qualities and conditions of the culture medium, and the design of the test vessel, shall be such as are shown to provide conditions favorable to aerobic and anaerobic growth of microorganisms throughout the test period.

(ii) *Ratio of inoculum to culture medium.* The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a

level that does not inhibit growth of contaminating microorganisms. Inhibitors or neutralizers of preservative may be considered in determining the proper ratio.

(f) *Exceptions.* Bulk and final container material shall be tested for sterility as described above in this section except as follows:

(1) *Different sterility tests prescribed.* When different sterility tests are prescribed for a product in this part.

(2) *Alternate incubation temperatures.* Two tests may be performed, in all respects as prescribed in paragraph (a)(1) of this section, one test using an incubation temperature of 18° to 22° C., the other test using an incubation temperature of 35° to 37° C., in lieu of performing one test using an incubation temperature of 30° to 32° C.

(3) *Different tests equal or superior.* A different test may be performed provided that prior to the performance of such a test a manufacturer submits data which the Director, National Institutes of Health, finds adequate to establish that the different test is equal or superior to the tests described in paragraphs (a) and (b) of this section in detecting contamination and makes the finding a matter of official record.

(4) *Test precluded or not required.* The tests prescribed in this section need not be performed for Whole Blood (Human), Cryoprecipitated Antihemophilic Factor (Human), Red Blood Cells (Human), Single Donor Plasma (Human), Smallpox Vaccine and other similar products concerning which the Director, National Institutes of Health, finds that the mode of administration, the method of preparation or the special nature of the product precludes or does not require a sterility test.

(5) *Viscous biological products.* Thioglycollate Broth Medium may be used in lieu of Fluid Thioglycollate Medium to test viscous biological products. The formula for Thioglycollate Broth Medium is as follows:

Thioglycollate Broth Medium. Certain biological products are turbid or otherwise do not lend themselves readily to culturing in Fluid Thioglycollate Medium because of its viscosity. In such instances, the following broth is acceptable in place of the Fluid Thioglycollate Medium, provided it is used in Smith fermentation tubes which have been heated within four hours in a boiling water bath or in free-flowing steam so as to drive the dissolved oxygen out of the medium in the closed arm:

L-cystine	0.5 gm.
Sodium chloride	2.5 gm.
($C_6H_{12}O_6 \cdot H_2O$) Dextrose	5.5 gm.
Yeast extract (water-soluble)	5.0 gm.
Pancreatic digest casein	15.0 gm.
Purified water	1,000 ml.
Sodium thioglycollate (or thioglycollic acid—0.3 ml.)	0.5 gm.

Final pH 7.1±0.1.

(6) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(7) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1±0.1, which shall be discharged into a final container by the same method used for filling the final containers with the product.

(8) *Samples—large volume of product in final containers.* For Normal Serum Albumin (Human), Normal Human Plasma, Antihemophilic Plasma (Human), Plasma Protein Solution (Human) and Fibrinogen (Human), when the volume of product in the final container is 50 ml. or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided,* That the containers and closures of the sample are identical with those used for the filling to which the test applies and the sample represents all stages of that filling.

(9) *Diagnostic products not intended for injection.* For diagnostic products not intended

for injection, (i) only the Thioglycollate Medium test is required; (ii) the volume of material for the bulk test shall be no less than 2.0 ml., and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

(10) *Human immune globulin preparations.* For human immune globulin preparations, the test samples from the bulk material and from each final container need be no more than 2.0 ml.

§ 73.740 Purity.

Products shall be free from extraneous material. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and other volatile substances.

(1) *Procedure.* The test for dried products shall consist of measuring the maximum loss of weight in a weighed sample equilibrated over anhydrous P_2O_5 at a pressure of not more than one mm. of mercury, and at a temperature of 20° to 30° C. for as long as it has been established is sufficient to result in a constant weight.

(2) *Test results; standard to be met.* The residual moisture and other volatile substances shall not exceed 1 percent except that for BCG Vaccine they shall not exceed 1½ percent, for Measles Virus Vaccine, Live, Attenuated, Measles-Smallpox Vaccine, Live, Rubella Virus Vaccine, Live and Antihemophilic Factor (Human), they shall not exceed 2 percent, and for Modified Plasma (Bovine); Thrombin; Fibrinogen; Streptokinase; Streptokinase-Streptodornase; and Anti-Influenza Virus Serum for the Hemagglutination Inhibition Test, they shall not exceed 3 percent.

(b) *Test for pyrogenic substances.* Each lot of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits as provided in subparagraphs (1) and (2) of this paragraph; *Provided,* That notwithstanding any other provision of this part, the test for pyrogenic substances is not required for the following products: Products containing formed blood elements; Cryoprecipitated Antihemophilic Factor (Human); Single Donor Plasma (Human); Normal Horse Serum; Normal Rabbit Serum; bacterial, viral and rickettsial vaccines and antigens; toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

(1) *Test dose.* The test dose for each rabbit shall be at least 3 milliliters per kilogram of body weight of the rabbit and also shall be at least equivalent proportionately, on a body weight basis, to the maximum single human dose recommended, but need not exceed 10 ml. per kilogram of body weight of the rabbit, except that: (i) Regardless of the human dose recommended, the test dose per kilogram of body weight of each rabbit shall be, at least 1 milliliter for immune globulins derived from human blood, at least 3 milliliters for Normal Human Plasma, and at least 30 milligrams for Fibrinogen (Human); (ii) for Streptokinase, Streptokinase-Streptodornase, Aggregrated Radio-Iodinated (I^{131}) Albumin (Human), Radio-Chromated (Cr^{51}) Serum Albumin (Human), Radio-Iodinated (I^{125}) Serum Albumin (Human) and Radio-Iodinated (I^{131}) Serum Albumin (Human), the test dose shall be at least equivalent proportionately on a body weight basis to the maximum single human dose recommended.

(2) *Procedure.* Products shall be tested for freedom from pyrogenic substances by intravenous injection of the test dose into three or more rabbits in overt good health and by recording for each rabbit a control temperature taken within one hour prior to injection, and three additional temperatures taken one, two, and three hours after injection. For purposes of subparagraph (3) of this paragraph, if there is no temperature increase over the control temperature (i.e. where the temperature remains unchanged or falls), the temperature rise shall be considered as zero. If there is an increase in temperature over the control temperature, the temperature rise shall be the difference between the highest of the three hourly

readings and the control temperature reading.

(3) *Test results; standards to be met.* The results recorded for all rabbits used in all tests of a lot of a product shall be included in determining whether the standard for purity is met. The product fails to meet test requirements if one half or more of all rabbits show a temperature rise of 0.6° C. or more or if the average temperature rise of all rabbits is 0.5° C. or more.

(c) *Different tests equal or superior.* A different test for residual moisture may be performed provided that prior to its performance the manufacturer submits data which the Director, National Institutes of Health, finds adequate to establish that the different test is equal or superior to the test described in paragraph (a) of this section and makes the finding a matter of official record.

§ 73.750 Test for *Mycoplasma*.

Except as provided otherwise in this part, prior to clarification or filtration in the case of live virus vaccines produced from in-vitro living cell cultures, and prior to inactivation in the case of inactivated virus vaccines produced from such living cell cultures, each virus harvest pool and control fluid pool shall be tested for the presence of *Mycoplasma*, as follows:

Samples of the virus for this test shall be stored either (1) between 2° and 8° C. for no longer than 24 hours, or (2) at −20° C. or lower if stored for longer than 24 hours. The test shall be performed on samples of the viral harvest pool and on control fluid pool obtained at the time of viral harvest, as follows: No less than 2.0 ml. of each sample shall be inoculated in evenly distributed amounts over the surface of no less than 10 plates of at least two agar media. No less than 1.0 ml. of sample shall be inoculated into each of four tubes containing 10 ml. of a semisolid broth medium. The media shall be such as have been shown to be capable of detecting known *Mycoplasma* and each test shall include control cultures of at least two known strains of *Mycoplasma*, one of which must be *M. pneumoniae*. One half of the plates and two tubes of broth shall be incubated aerobically at 36° C. ±1° C. and the remaining plates and tubes shall be incubated anaerobically at 36° C. ±1° C. in an environment of 5–10 percent CO_2 in N_2. Aerobic incubation shall be for a period of no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than 4 additional plates and incubated aerobically. Anaerobic incubation shall be for no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than four additional plates and incubated anaerobically. All inoculated plates shall be incubated for no less than 14 days, at which time observation for growth of *Mycoplasma* shall be made at a magnification of no less than 300×. If the Dienes Methylene Blue-Azure dye or an equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of *Mycoplasma*. The presence of the *Mycoplasma* shall be determined by comparison of the growth obtained from the test samples with that of the control cultures, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for vaccine manufacture if none of the tests on the samples show evidence of the presence of *Mycoplasma*.

§ 73.760 Identity.

The contents of a final container of each filling of each lot shall be tested for identity after all labeling operations shall have been completed. The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or in vitro or in vivo immunological tests.

§ 73.770 Requests for samples and protocols; official release.

Samples of any lot of any licensed product, together with the protocols showing results of applicable tests, may at any time be required to be sent to the Director, Division of Biologics Standards. Upon notification by the Director, Division of Biologics Standards, a manufacturer shall not distribute a lot of a product until the lot is released by the Director, Division of Biologics Standards: *Provided*, That the Director shall not issue such notification except when deemed necessary for the safety, purity or potency of the product.

§ 73.780 Cultures.

(a) *Storage and maintenance.* Cultures used

CLINICAL EVALUATION OF AL-1067

ANTIHEMOPHILIC FACTOR, HUMAN

Introduction:

Concentrates of Antihemophilic Factor (AHF, AHG, Factor VIII) have been demonstrated to be effective in the temporary replacement of that coagulation factor in patients with Hemophilia A, a congenital deficiency of Factor VIII. Such concentrates of AHF range from cryoprecipitate and Fraction I of Cohn to highly purified preparations representing as much as a hundred fold concentration of the level of AHF in normal plasma. The need for concentrates of AHF is due to the fact that excessively large volumes of normal plasma are required to control bleeding in patients undergoing surgery or suffering traumatic hemorrhage.

Methods of extraction of AHF activity from cryoprecipitate lead to a solution containing, in addition to Factor VIII, part of the fibrinogen and of other plasma constituents entrapped in the cryoprecipitate. Selection of the method of processing is therefore based on a balance between the degree of purification desired and a maximum yield of antihemophilic factor activity.

Effectiveness in elevating Factor VIII levels is regarded as directly related to the total amount of AHF activity infused and independent of the state of purification. Other factors such as infusion volume, protein concentration, stability, incidence of reactions, clarity of solution, and convenience of administration must be considered in establishing the extent of purification desired.

AL-1067 represents an AHF concentrate in the intermediate range of potency, 6-10 AHF units per ml. after reconstitution with distilled

water, and is designed to meet the tentative standards for Antihemophilic Factor (Human). Clinical evaluation was planned to assure that the AHF activity, as determined in vitro, was effective in vivo. Also, freedom from adverse effects, such as pyrogenicity and transfusion reactions, was assessed. General comparisons of AL-1067, with regard to convenience in administration and patients acceptability were made by the individual investigator in the light of his experience with other experimental and commercial AHF concentrates.

Clinical evaluation of six batches of AL-1067 was carried out by three investigators. The investigators and their affiliations are shown below:

1. Charles F. Gehrke, M.D. in collaboration with John Penner, M.D., Dept. of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan.
2. George R. Honig, M.D., Assoc. Prof., Dept. of Pediatrics, University of Illinois College of Medicine, Chicago, Illinois College of Medicine, Chicago, Illinois.
3. Jack Lazerson, M.D., Chief of Hemophilia Service, Childrens Hospital at Stanford, in collaboration with Judith Pool, Ph.D., Dept. of Medicine, Stanford University School of Medicine, Palo Alto, California.

Methods:

1. Protocol

Each investigator accepted protocol #MC072971, see Appendix A, as the guide for his study. Modifications in protocol in each study

were those that were compatible with standard procedure at the specific institution in regard to treatment of Hemophilia A. Results were reported by means of a patient record supplied by the sponsor and photocopies are supplied with this report.

2. Materials

Clinical supplies of AL-1067 consisted of unit packages, each containing a vial of Antihemophilic Factor, lyophilized; a vial of Water for Injection, U.S.P., 25 ml.; a double-end needle for reconstitution; and an intravenous administration set. The unit packages were stored at refrigeration temperatures (2-8° C.).

In Table I are shown the batches of AL-1067 that were tested clinically and pertinent data regarding them. It should be noted that three batches were prepared by Method A in which glycine is used to precipitate AHF activity. The other three batches were made by Method B described further in this application and employed in subsequent batches. All batches are representative of the methods to be used in collection of plasma and preparation of cryoprecipitate. They differ in the addition of heparin to the extraction buffer (Method B) to prevent activation of any residual prothrombin not adsorbed by aluminum hydroxide. Procedures involved in preparation of the final package, sterile filtration and lyophilizing were identical in all six clinical batches. No clinical differences were detectable between batches and all were considered to be entirely satisfactory by the investigators.

Administration of the reconstituted solution was, by choice of the investigator, either by syringe or by infusion through the administration set provided.

TABLE I
SUMMARY OF CHEMICAL DATA
ON AL-1067 USED IN CLINICAL STUDIES

Preparation				AHF	Protein						
Batch No.	Date	Method	Size *	Activity u/vial	Total mg/vial	Total mg/unit	Clottable mg/vial	Clottable mg/unit	AL-HF p.p.m.	Heparin u/vial	Evaluated by
K499096	4/30/71	A	100	132	360	2.7	315	2.4	< 0.7	---	Dr. Lazerson
K499104	5/19/71	A	100	198	570	2.9	375	1.9	< 0.8	--	Dr. Gehrke
K499105	5/ 7/71	A	100	278	495	1.8	337	1.2	< 0.7	--	Drs. Gehrke, Lazerson
K499129	11/17/71	B	50	187	720	3.8	398	2.1	< 0.7	17	Dr. Honig
K499131	11/16/71	B	100	184	780	4.2	520	2.8	< 0.7	19	Dr. Lazerson
K499137	11/17/71	B	300	208	765	3.7	515	2.5	< 0.7	13	Dr. Gehrke

*Liters of plasma in the starting material.

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PROTOCOL FOR CLINICAL EVALUATION OF
AL-1067 (ANTIHEMOPHILIC FACTOR, HUMAN)
IN HEMOPHILIA A
(Protocol No. MC072971)

I. PURPOSE

To evaluate the safety and effectiveness of AL-1067 as a concentrate of Antihemophilic Factor from normal human plasma.

II. PATIENT SELECTION

Patients previously diagnosed as deficient in Factor VIII (antihemophilic globulin) and in need of prophylactic or therapeutic replacement of Factor VIII are regarded as suitable candidates for study.

III. PATIENT EXCLUSIONS

Patients with abnormal liver or kidney function are excluded from study.

IV. NUMBER OF PATIENTS AND DURATION OF TREATMENT

Not more than 15 patients are to be treated. Duration of treatment is not to exceed 10 days.

V. GENERAL PLAN OF STUDY

A. Prophylaxis. Patients exhibiting low levels of circulating Factor VIII will be treated with sufficient AL-1067 to increase the circulating level of Factor VIII to approximately 50% of normal. Partial thromboplastin time and fibrinogen levels will be determined before and at intervals during the first [illegible] hours after AL-1067 infusion. Other laboratory tests of pre- and the 24-hour post-treatment blood samples will include:

1. Complete blood count
2. Bilirubin
3. Haptoglobin
4. Coombs test.

Patients will be observed during the test period for signs or symptoms indicative of any adverse effects. Tests may be repeated in selected patients for the purpose of confirming results or for assessing the presence of pre-existing AHF inhibitors.

B. Therapy. Patients in need of Factor VIII to control hemorrhage may be treated with AL-1067 for that purpose. Such evaluation will be made only after the investigator is satisfied with the safety and effectiveness of AL-1067 under section A above.

VI. ADMINISTRATION AND DOSAGE

AL-1067, reconstituted with water for injection as provided, is intended only for intravenous administration.

Dosage for prophylaxis: For an increase of 1% of the normal level desired administer 0.5 units AHF per kilogram of body weight. (Example: for a child weighing 20 Kg. an increase from 20% to 40% of normal would require 0.5 x 20 x 20 or 200 AHF units.)

Dosage for treatment of overt bleeding: Initially, 20 units per Kg. of body weight; after eight hours, 10 units per Kg. Further treatment with 10 units per Kg. at eight hour intervals will depend upon the extent of bleeding and the need to establish further control.

VII. PATIENT RECORD FORMS

Completed reports for each patient on forms provided by the Company are to be forwarded to the Clinical Research Department, Armour Pharmaceutical Company, Kankakee, Illinois, following conclusion of the study.

VIII. DISCONTINUATION OF TREATMENT

Appearance of signs or symptoms that could, in the opinion of the investigator, place the welfare of any patient in jeopardy, shall constitute grounds for discontinuation of AL-1067 treatment. Further, the patient is to be followed and examined by the investigator until the signs or symptoms have subsided.

Armour Pharmaceutical Co.
Clinical Research Department
MC072971

Results:

Details of individual studies by each investigator are described below.

(A.) Evaluation of AL-1067 by Dr. Gehrke

The study consisted of fifteen treatments of eleven patients with established Hemophilia A. Dosage of AL-1067 was 10 AHF units per pound of body weight (22 u/Kg.) which was expected to provide an increase in Factor VIII of 35 to 50% of the normal level immediately after injection.

Patients ranged in age from 13 to 35 years, and in body weight from 120 to 200 lbs. All were part of an ongoing program of prophylactic therapy and were given AL-1067 in place of AHF concentrates prepared by the Michigan State Department of Health. In none was there hemorrhage calling for immediate therapeutic administration of AHF. Thus, the investigators did not attempt to assess clinical benefit but limited their evaluation to the effects on laboratory parameters and to observations for possible side effects. Details are presented in Table II. Comments on each trial as provided by Dr. Gehrke are attached, Appendix B; and copies of patient record forms.

TABLE IV

RESULTS OF CLINICAL STUDY OF AL-1067 (DR. GERRIS)

SUBJECT	1	10	2	3	4	5	6	15	7	8	9	12	11	13	14
Patient	GRO-A					GRO-A						GRO-A			
Date of Trial	9-6-71	9-10-71	9-9-71	9-20-71	9-26-71	9-27-71	9-30-71	1-19-72	9-30-71	10-18-71	10-18-71	11-29-71	11-11-71	12-1-71	1-18-72
Age	35	35	24	24	25	26	30	30	15	26	13	13	19	19	31
Wt. (Kg.)	91	91	68	85	84	59	59	61	55	73	91	95	64	64	57
AHF Units	2140	1904	1508	1866	1824	1268	1268	1458	1268	1548	2034	2080	1390	1664	1248
U/kg.	23.5	21	22	22	22	22	22	24	23	21	23	22	22	26	22
Volume, ml.	250	200	150	175	250	150	150	175	150	175	200	250	125	160	150
Infusion Time, Min.	20	10	15	5	15	10	8	15	5	8	10	10	5	7	15
Via	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe
% Factor VIII, In.	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	1	< 1	< 1	< 1	< 1	< 1	1
" , Imm.	50	8	23	25	< 1	< 1	26	100	23	33	< 1	< 1	26	33	100
" , 2 hr.	25	-	16	15	< 1	< 1	24	29	10	23	< 1	< 1	20	-	32
" , 24 hr.	8	< 1	7	1	< 1	< 1	1	1	1	6	< 1	< 1	7	< 1	1
PT Time (Sec.), In.	139.9	124.4	93.8	69.4	88.0	78.9	92.2	> 180	63.9	97.9	83.9	150	94.3	> 180	61.6
" , Imm.	44.7	54.7	44.9	31.4	102.1	92.9	38.8	26.8	36.8	33.6	75.7	150	48.9	35.7	32
" , 2 hr.	43.3	-	51.9	33.0	95.0	70.6	47.2	42.6	41.8	38.7	70.3	150	43.3	-	38
" , 24 hr.	57.6	62.1	66.5	70.0	111.6	81.9	50.1	51.2	58.5	54.9	79.5	> 180	59.4	51.8	57.2
Pro. Time (Sec.), In.	11.5	14.7	11.1	11.6		12.1	11.4	10.6	10.5	12.6	11.8	10.7	11.5	12.0	11.1
" , Imm.	11.3	14.9	10.9	11.1		12.7	10.5	10.6	10.4	13.6	11.8	10.6	11.1	11.6	10.5
" , 2 hr.	11.3	-	-	11.8	12.0	11.7	11.4	10.4	10.6	10.9	10.5	10.5	10.9	-	10.5
" , 24 hr.	11.6	11.7	11.2	11.2	10.6	11.7	11.2	10.6	11.7	11.5	11.3	10.7	11.4	10.9	10.6
Hemoglobin, mg.%, In.	16.5	16.8	16.1	16.0	15.3	14.0	15.5	16.9	-	-	16.8	15.8	15.1	15.6	14.9
" , 24 hr.	15.4	15.4	15.8	14.9	14.7	13.4	14.1	16.7	-	-	-	16.8	14.5	14.9	14.8
Hematocrit, %, In.	47	49	44.5	46	45.5	39.5	45	51	-	-	48	45.5	44	44	43
" , 24 hr.	43.5	46.5	43.5	45	46.5	41.5	42.5	47	-	-	-	47.0	41.5	43	44
Haptoglobin, mg.%, In.	1-200	< 20	< 20	20-50	< 20	< 20	< 20	< 20	-	20-50	20-50	20-50	100-200	20-50	20-50
" , 24 hr.	20-50	50-100	< 20	50-100	< 20	< 20	< 20	20-50	-	-	-	20-50	100-200	20-50	20-50
SGOT, u., In.	100	98	44	57	21	66	70	66	-	55	60	76	82	63	56
" , 24 hr.	63	107	48	47	50	64	70	49	-	-	-	59	48	73	66
Previous AHF Therapy															
Days before AL-1067	5		3 1/2 wk.	2	4 wk.	3	6		1 wk.	11			6		
Units	1235		1000	936	3500	2000	?		1500	1600			3000		
Clinical Results		Good											Good		
Adverse Effects	None	*	None	None	None	None	None	None	None	None	None	None	None	None	None
AL-1067: Vials															
Lot K499104	8	4	2	1	5	5	5	-	5	5	2	-	-	-	-
Lot K499105	2	4	4	6	3	1	1	-	1	2	6	-	5	-	-
Lot K499137	-	-	-	-	-	-	-	7	-	-	-	10	-	8	6

*Some "full feeling" in head during infusion.

APPENDIX B

REPORT ON ANTIHEMOPHILIC FACTOR (HUMAN)
BY DR. GEHRKE

ARMOUR PHARMACEUTICAL -- Report on Antihemophilic Factor (Human)

Protocol AL 1067

Antihemophilic factor (Lot AL 1067) was employed to treat patients with classical hemophilia (Factor VIII deficiency). A dose of 10 units per pound was administered on eleven occasions to eleven patients. Laboratory values were obtained before, immediately after and at 2 and 24 hours post infusion. Studies were obtained to both assess the Factor VIII activity of the infused material as well as to evaluate any possible side effects, and included measurement of antihemophilic factor activity, partial thromboplastin time, prothrombin time, hemoglobin, hematocrit, serum transaminase and serum haptoglobin.

The highest factor VIII level attained was 50% immediately post infusion, and the highest level of activity 24 hours post infusion was 8%. Four patients were noted to have an inhibitor against factor VIII and failed to develop a significant rise in the factor VIII levels. A fall in hematocrit 24 hours following infusion of the antihemophilic material was observed in four patients. Haptoglobin levels were low prior to the therapy as a result of hemorrhage and the red cell breakdown. No other significant laboratory changes were noted and clinical evidence of hepatitis was not noted.

In addition, five patients were treated at the Hemophilia Camp this summer prior to the evaluation with the larger group. Each of these patients received a single bottle of the product (250 to 300 units). There was no significant rise in their factor activity either immediately following infusion or at any time thereafter. The infusion was tolerated well by all of the boys except one who developed a fever to 101° within 12 hours after receiving the material. The fever persisted for an additional 24 hours and then subsided spontaneously. There was no evidence of hemolysis nor evidence of hepatitis in any of the boys who received this material.

Subject #1. 35 year old, 200 lb. male with a well established diagnosis of classical hemophilia with less than 1% factor VIII activity. He received 2,140 units of factor VIII material in 250 cc of fluid over a period of 20 minutes. His factor VIII activity rose to 50% 5 minutes following completion of the infusion and was at 25% 2 hours later, and 8% at 24 hours. His hematocrit was 47 just

prior to infusion, fell to 43.5 vols% 24 hours post infusion. The haptoglobin was 100-200 mg% prior to infusion, fell to 20-50 mg% 24 hours post infusion. The PTT was 139.9 seconds prior to infusion and fell to 44.7 seconds immediately following infusion and was 57.6 seconds 24 hours after infusion.

Subject #2. 24 year old hemophiliac weighing 150 lb. received 1,508 units of AHF in 150 cc over a 15 minute period of time. His factor VIII activity rose to 23% immediately post infusion and was 7% 24 hours following infusion. The PTT was 93.8 seconds prior to infusion, 44.9 seconds immediately following and 65.5 seconds 24 hours after infusion. His hemoglobin and hematocrit remained essentially stable over the 24 hour period following infusion. No adverse reactions were noted.

Subject #3. A 24 year old hemophiliac weighing 187 lbs. In addition to his hemophilia, he is under treatment for idiopathic epilepsy with phenobarbitol 32 mg 4 tid and mysoline 250 mg tid. He received 1,866 units of AHF in 175 cc fluid over a 5 minute period of time. His factor VIII activity prior to infusion was less than 1%, rose to 25% immediately after infusion and 1% activity at 24 hours. His PTT was 69.4 seconds prior to infusion, 31.4 seconds immediately after and 70.3 seconds at 24 hours. His hematocrit remained stable as did his haptoglobin levels. No adverse reactions were noted.

Subject #4. A 25 year old hemophiliac weighing 185 lbs. received 1,824 units in 200 cc fluid over a 15 minute period of time. His factor VIII activity was less than 1% prior to infusion and remained at less than 1% throughout the study. His PTT was 88 seconds prior to infusion and remained elevated at 102 seconds immediately following infusion and 111.6 24 hours later. No adverse side effects were noted. His hemoglobin and hematocrit remained stable.

Subject #5. 26 year old hemophiliac weighing 130 lb. received 1,268 units of AHF in 150 cc fluid over a 10 minute period of time. His factor VIII activity was less than 1% prior to infusion and remained at this level throughout the 24 hour period. His PTT was 72.9 seconds prior to infusion, 92.9 seconds immediately after infusion and was 81.9 seconds 24 hours post infusion. His hemoglobin and hematocrit remained stable as did his other laboratory work. No adverse side effects were noted.

Subject #6. 30 year old hemophiliac weighing 130 lbs. received 1,268 units of AHF material in 150 cc fluid over an 8 minute period of time. His factor VIII activity was less than 1% prior to, 26% immediately after and 1% 24 hours after infusion. His PTT was 92.2 seconds prior to, 38.8 seconds following and 50.1 seconds after infusion. His hematocrit was 45 vols% prior to and 42.5 24 hours after infusion. No adverse side effects were noted.

Subject #7. 15 year old hemophiliac weighing 120 lb. received 1,268 units of factor VIII material in 150 cc of fluid over a 5 minute period of time. Factor VIII activity was 1% prior to infusion, 23% immediately after and 1% 24 hours after infusion. His PTT was 63.9 seconds prior to infusion, 36.8 seconds immediately after and 58.5 seconds 24 hours after infusion. No adverse side effects were noted.

Subject #8. 26 year old hemophiliac weighing 160 lb. received 1,548 units of factor VIII material in 175 cc fluid over an 8 minute period of time. His factor VIII activity was less than 1% prior to infusion, 33% immediately after and 6% at 24 hours. His PTT was 97.9 seconds prior to infusion, 33.6 seconds immediately after, 54.9 seconds 24 hours post infusion. No adverse side effects were noted.

Subject #9. 13 year old hemophiliac weighing 200 lbs. received 2,064 units of factor VIII material in 200 cc fluid over a 10 minute period of time. His factor VIII activity was less than 1% prior to infusion and did not rise above this level throughout the 24 hour study period. His PTT was 83.9 seconds prior to infusion, 75.7 immediately after and 71.5 seconds at 24 hours. No adverse reactions were noted.

Subject #10. 35 year old hemophiliac weighing 200 lbs received 1,904 units of AHF in 200 cc fluid over a 10 minute period of time. His factor VIII activity was less than 1% immediately prior to infusion, 8% after infusion and 1% at 24 hours. The patient complained of a "full feeling" in his head during infusion, but no other difficulties were encountered. His hematocrit prior to infusion was 49 vol% and 46.5 vol% 24 hours later. The haptoglobin level was less than 20 mg% prior to infusion and was 50-100 mg% at 24 hours.

Subject #11. 19 year old hemophiliac weighing 140 lbs received 1,390 units of factor VIII in 125 cc of fluid over a 5 minute period of time. This patient is taking Potaba 18 capsules per day (500 mg each). His factor VIII activity was less than 1% prior to infusion, 26% immediately after and 7% 24 hours later. His PTT was 94.3 seconds prior to infusion, 48.9 seconds immediately after and 59.4 at 24 hours. His hematocrit was 44 vols% prior to infusion and was 41.5 vols% 24 hours lat

GRO-C

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Addendum to:

ARMOUR PHARMACEUTICAL -- Report on Antihemophilic Factor (Human)

Protocol AL 1067

Antihemophilic factor (Lot AL 1067) was employed to treat patients with classical hemophilia (Factor VIII deficiency). A dose of 10 units per pound was administered to 4 hemophilic patients. Laboratory values were obtained immediately prior to injection, immediately following and at 2 and 24 hour intervals after infusion.

Studies were obtained to assess the Factor VIII activity and any possible side effects. The laboratory measurements included antihemophili factor activity (Factor VIII), partial thromboplastin time, prothrombin time, hemoglobin, hematocrit, serum transanimase and serum haptoglobin.

Two of the boys attained levels of 100% Factor VIII activity immediately following injection of the antihemophilic factor. All of the boys had returned to 1% factor activity or less at the end of 24 hours. One patient was noted to have an inhibitor to Factor VIII and failed to show any increase in his Factor VIII activity either immediately or 2 hours after infusion. There were no significant abnormalities noted in the other laboratory studies. Several of the boys had a decreased haptoglobin level prior to infusion and these were unchanged 24 hours after infusion. There were no significant changes in hemoglobin or hematocrit levels 24 hours after infusion.

All of the boys tolerated treatment well and there were no subjective side effects.

Subject #12. 13 yr. old 200 lb. hemophiliac received 2,080 units of AHF via syringe injection over a 15 to 20 minute period. There was no demonstrable rise in his Factor VIII activity nor was there any decrease in his partial thromboplastin time at any time following infusion. This patient was felt to have an inhibitor to Factor VIII.

Subject #13. 19 yr. old 140 lb. hemophiliac received 1,640 units of AHF in 160 cc fluid over 7 minutes via syringe injection. His Factor VIII activity rose to 33% from a pre-treatment level of less than 1%. His partial thromboplastin time fell from a pre-treatment level of greater

than 3 minutes to a level of 35.7 seconds immediately after infusion. At 24 hours his Factor VIII activity was less than 1% and the partial thromboplastin time had risen to 51.8 seconds. His hemoglobin and hematocrit as well as his haptoglobin levels remained stable for the 24 hours following infusion. The patient had no subjective difficulties with the infusion.

Subject #14. 31 yr. old 125 lb. hemophiliac received 1,248 units of factor VIII material in 150 cc over 15 minutes through a syringe. His Factor VIII activity just prior to infusion was 1% and his partial thromboplastin time 61.6 seconds. Immediately after infusion his Factor VIII activity was 100% and his partial thromboplastin time was 32 seconds. At 24 hours his Factor VIII activity had fallen to 1% and his partial thromboplastin time had risen 57.2 seconds. There were no changes in his hematocrit or hemoglobin levels. He had no subjective complaints.

Subject #15. 30 yr. old 135 lb. hemophiliac received 1,458 units in 150 cc of fluid over 15 minutes through a syringe. His pre-treatment level of Factor VIII was less than 1%, his partial thromboplastin time had fallen to 26.8 seconds. Twenty-four hours after infusion his Factor VIII activity was 1% and his partial thromboplastin time had risen to 51.2 seconds. There were no changes in his hemoglobin and hematocrit levels. The patient had no subjective complaints.

GRO-C

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January 25, 1972

(B.) Evaluation of AL-1067 by Dr. Lazerson

Dr. Lazerson conducted 17 trials in 13 patients ranging in age from 6 to 26 years and in weight from 19 to 70 Kgs. All were patients with established Hemophilia A and most of them are involved in a program of self-administration conducted by the Hemophilia Service, Children's Hospital at Stanford. Cryoprecipitate, prepared by several blood banks in the area, is the product generally used in the program but concentrates of antihemophilic factor are available to each patient for emergency purposes or for use when the patient is away from home.

AL-1067 was evaluated for its effectiveness in increasing the circulating level of Factor VIII following administration of about 20 units per kilogram of body weight. During the first eight trials, samples were drawn immediately and every hour for the first four hours after administration of AL-1067. A 24-hour post-infusion sample was also taken. Factor VIII levels were determined by the method of Pool and Robinson (Brit. J. Haemat. 5:17, 1959), employing a reference curve based on values obtained from a pool of normal human plasma. Partial thromboplastin times were also determined. Under the conditions of this test, the normal range is 25-45 seconds. The coagulation tests were conducted by Dr. Judith Pool, Dept. of Medicine, Stanford University. Patients were observed for possible side effects and blood pressure and body temperature measurements were taken frequently.

In later trials, when the investigator was satisfied with safety and efficacy considerations, measurements were limited to pre- and immediate post-infusion measurements of Factor VIII. One patient, GRO-A, was

known to have a low level of Factor VIII inhibitor. Two patients, GRO-A and GRO-A, received cryoprecipitate either shortly before or before and after AL-1067 for treatment of hemarthroses.

The first trial of AL-1067 in patient GRO-A was made during post-synovectomy while the patient was hospitalized and was receiving cryo-precipitate and epsilon amino caproic acid (Amicar) 3 gm. every six hours. The patient received ten units of cryoprecipitate again 15 hours after AL-1067. The infusions of cryoprecipitate account for the elevated Factor VIII levels in the initial and post-Rx samples. Later, when AL-1067 was again infused, patient GRO-A developed hives which were relieved by oral administration of Benadryl, 50 mg. This was the only side effect reported in connection with the trials of AL-1067.

Dr. Lazerson was well satisfied with the performance of AL-1067. He and his associates were particularly well impressed with its ease of reconstitution and the clarity of the reconstituted solution. Data are summarized in Table III.

TABLE III

RESULTS OF CLINICAL STUDY OF AL-1067 (DR. LAZERSON)

Patient	GRO-A				GRO-A						GRO-A						
Date of Trial	6-17-71	6-17-71	6-17-71	6-17-71	12-10-71	6-30-71	12-10-71	8-11-71	8-11-71	8-11-71	11-30-71	12-9-71	12-1-71	12-8-71	12-8-72	12-17-71	12-20-71
Age	8	6 1/2	8	16	16	16	16	16	14	15	15	15	9	17	15	26	15
Wt. (Kg.)	21	19	27	73	75	52	53	44	40	70	67	67	50	65	52	55	49
AHF units	395	410	542	1600	1288	1244	920	1004	924	1800	1104	1104	736	1104	1104	920	920
U/Kg.	19	21	20	25	17	24	17	25	23	26	17	17	15	17	21	17	19
Volume, ml.	75	50	100	175	175	125	125	150	175	175	150	150	100	150	150	125	125
Infusion Time, Min.	12	5	7	25	18	3	15	14	14	15	17	13	20	16	16	21	11
Via	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set IP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP	IV Set OP
% Factor VIII, In.	< 1	< 1	< 1	< 1	< 1	37	< 1	< 1	1.2	< 1	< 1	< 1	< 1	< 1	4	7	< 1
" , Imm.	26	25	25	22	41	54	36	26	39	35	53	55	23	39	56	48	41
" , 1 hr.	20	18	23	18	-	55	-	23	41	29	-	-	-	-	-	-	-
" , 2 hr.	19	16	18	15	-	42	-	18	32	21	-	-	-	-	-	40	-
" , 3 hr.	15	-	15	10	-	44	-	18	32	21	-	-	-	-	-	40	-
" , 4 hr.	16	14	14	-	-	42	-	17	23	20	-	-	-	-	-	-	-
" , Post-Rx	5	3	4	3	-	42	-	4	8	4	-	-	-	-	-	-	-
P.T. Time (Sec.), In.	80.5	78.0	81.2	81.0	-	47.5	-	-	-	-							
" , Imm.	61.5	53.8	60.0	56.3	-	47.3	-	-	-	-							
" , 1 hr.	54.3	56.5	57.5	55.7	-	39.0	-	-	-	-							
" , 2 hr.	58.5	55.3	59.0	61.6	-	-	-	-	-	-							
" , 3 hr.	57.5	-	60.0	59.0	-	-	-	-	-	-							
" , 4 hr.	56.3	58	54.5	-	-	42.5	-	-	-	-							
" , Post-Rx	61.0	60.3	56.0	71.5	-	46.3	-	-	-	-							
" , Control	35.5																
B.P., In.	110/60	100/64	100/58	112/70	118/70	118/70	114/65	112/70	118/78	132/72	115/70	122/80	120/72	122/75	110/70	125/75	125/68
" , Imm.	100/60	100/60	94/60	110/80	120/72	116/70	112/70	110/70	120/80	132/70	115/70	118/80	110/72	120/70	110/74	120/75	126/70
" , 1 hr.	100/60	90/60	90/60	110/74	-	120/68	-	114/70	120/70	130/74	-	-	-	-	-	-	-
" , 2 hr.	100/60	98/58	94/60	110/70	-	120/70	-	120/65	116/70	130/70	-	-	-	-	-	-	-
" , 3 hr.	100/60	-	98/60	114/70	-	-	-	110/65	120/70	128/70	-	-	-	-	-	122/72	-
" , 4 hr.	102/60	100/60	98/56	-	-	110/70	-	110/70	120/70	130/70	-	-	-	-	-	-	-
" , Post-Rx	105/65	54/62	100/60	110/70	-	115/70	-	110/68	120/74	128/70	-	-	-	-	-	-	-
Temperature, In.	37.2	37.2	37.2	36.8	37.2	36.4	37.0	37.7	37.4	36.8	37.8	37.2	36.8	37.8	37.2	37.6	37.0
" , Imm.	36.8	37.4	37.4	37.2	37.2	36.8	37.0	37.9	37.4	37.0	37.8	37.2	37.0	37.8	37.4	37.4	37.2
" , 1 hr.	37.0	37.0	36.9	37.0	-	37.4	-	37.4	37.8	37.2	-	-	-	-	-	-	-
" , 2 hr.	37.0	37.2	37.8	36.8	-	37.8	-	37.1	37.4	36.8	-	-	-	-	-	-	-
" , 3 hr.	37.2	-	37.0	36.8	-	37.0	-	38.0	37.2	36.8	-	-	-	-	-	37.4	-
" , 4 hr.	37.6	37.8	37.0	-	-	37.2	-	37.2	37.4	37.0	-	-	-	-	-	-	-
" , Post-Rx	37.2	37.2	37.2	37.0	-	37.6	-	37.6	37.4	37.0	-	-	-	-	-	-	-
PCV, %	38	37	35	38	43	-	41	41	40	46	49	49	39	43	40	46	40
Clinical Response	OK	OK	OK	OK	-	Good	-	OK	OK	OK	-	-	Good	Good	Good	-	Good
Adverse Effects	None	None	None	None	None	None	Hives	None	None	None	None	None	None	None	None	None	None
AL-1067: Vials																	
Lot M499096	3	1	2	1	-	1	-	4	7	1	-	-	-	-	-	-	-
Lot M499105	-	1	1	6	-	4	-	2	-	6	-	-	-	-	-	-	-
Lot M499131	-	-	-	-	7	-	5	-	-	-	6	6	4	6	6	5	5

(c.) Evaluation of AL-1067 by Dr. Honig

Patients with established hemophilia presenting themselves to the clinic for treatment of acute hemarthrosis were the subjects of this study. Seven patients ranging in age from 3 to 14 years (mean age: 9) and in weight from 16 to 64 Kg. (mean body weight: 32 Kg.) received AL-1067. One patient received a second infusion of AL-1067 two weeks after the first. A second patient received four doses in an effort to control hemorrhage.

Determinations of Factor VIII levels were made before and immediately after intravenous administration of Factor VIII and, in most cases, again at twenty four hours. Results as shown in Table IV indicate that dosages of AL-1067 were effective in the treatment of hemarthroses as judged clinically and as demonstrated by improvements in circulating levels of Factor VIII. Adverse effects were minimal. Fibrinogen levels were increased immediately after infusion and exceeded the normal range (150-400 mg.%) in three patients. At twenty-four hours levels had returned to or below initial values. Determinations of fibrinogen were made by the method of Ratnoff and Maizel, J. Lab. Clin. Med. 37:316, 1951. Haptoglobin determinations were disregarded because of hemolysis of samples. In six of seven patients clinical benefits were clear. In the seventh, difficulty in controlling hemorrhage (epistaxis) made evaluation of benefit less certain. In the opinion of Dr. Honig and his associates AL-1067 was entirely satisfactory as a concentrate of antihemophilic factor.

TABLE IV

RESULTS OF CLINICAL STUDY OF AL-1067 (DR. HONIG)

Patient	GRO-A									
Date of Trial	11-30-71	12-1-71	12-2-71	12-10-71	12-15-71	12-20-71	12-15-71	12-16-71	12-16-71	12-17-71
Age	7	7	7	14	14	7	9	9	3	3
Wt. (Kg.)	20	20	24	44	64	27	30	30	16	16
AHF Units	560	560	560	1120	1300	560	935	935	374	374
U/Kg.	28	28	24	25	20	21	31	31	24	24
Volume, ml.	75	75	75	150	175	75	125	125	50	50
Infusion Time, Min.	15	-	10	10	15	10	10	10	10	15
Via	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe	Syringe
Hospital or O.P.	OP	OP	OP	OP	OP	IP	IP	IP	OP	OP
Factor VIII, %, In.	< 1	< 1	< 1	< 1	1.8	3.7	< 1	9	< 1	< 1
" , Imm.	35	49	65	25	45	50	52	65	29	-
" , 2 hr.	-	-	-	-	-	-	-	-	22	-
" , 24 hr.	11	14	-	7	16	11	9	9	6	-
" , 72 hr.	-	-	-	-	-	-	-	-	< 1	-
P.T. Time (Sec.), In.	103.5	114.4	110.0	94.7	163.5	93.0	96.5	67.7	154.2	-
" , Imm.	46.5	48.7	53.6	46.8	66.0	61.5	75.0	50.5	60.9	-
" , 2 hr.	-	-	-	-	-	-	-	-	47.9	-
" , 24 hr.	59.0	62.0	-	69.5	53.4	80.0	67.7	51.2	77.0	-
" , 72 hr.	-	-	-	-	-	-	-	-	80.9	-
Pro. Time (Sec.), In.	12.7/11.0	11.4/10.4	12.2/10.5	12.0/11.0	10.4/10.4	13.0/12.0	11.5/10.5	11.5/10.9	11.9/10.4	-
" , Imm.	12.9/11.0	11.7/10.4	13.7/10.5	12.7/11.0	11.4/10.4	13.0/12.0	11.5/10.5	11.5/10.9	12.9/10.4	-
" , 2 hr.	-	-	-	-	-	-	-	-	12.9/10.4	-
" , 24 hr.	12.0/11.0	12.4/10.9	-	11.7/10.5	10.9/10.9	13.4/11.0	11.5/10.9	11.2/10.5	11.5/10.7	-
" , 72 hr.	-	-	-	-	-	-	-	-	10.4/10.5	-
Fibrinogen, mg.%, In.	424	284	468	308	288	-	900	-	236	-
" , Imm.	396	416	732	424	376	-	1028	-	328	-
" , 24 hr.	416	320	-	236	344	-	868	-	380	-
Fraction I, In.	1.8	1.4	1.7	1.5	-	-	-	-	0.9	-
" , Imm.	-	1.9	2.1	2.0	-	-	-	-	1.3	-
" , 24 hr.	1.3	-	-	-	-	-	-	-	1.2	-
Inhibitor, In.	Neg.	Neg.	-	Neg.	Neg.	-	-	-	Neg.	-
Clinical Response	Imp.	Imp.	Imp.	Imp.	Imp.	Imp.	Imp.	Imp.	*	*
Adverse Effects	None	None	None	None	None	None	None	None	None	None
AL-1067: Vials										
Lot K499129	3	3	3	6	7	3	5	5	2	2

*Patient GRO-A: Approximately 75 units AHF lost due to infusion difficulty 12/17/71. Prophylactic treatment repeated every three days to control severe recurrent epistaxis. Some improvement noted after fourth treatment.

verts histidine to imocanic acid. Urocanic acid is then converted by imocanase to α-formimino-L-glutamic acid, which is eventually broken down to glutamic acid, formic acid and ammonia. The presence of methylhistidine in the blood and urine of the 2 patients implies that this metabolic pathway for histidine is open. We have no data on the children's histamine metabolism. The present findings could be explained by a deficiency of either histidine α-deaminase or urocanase. By analogy with phenylketonuria, one would expect not only increased amounts of the amino acid in the urine but also other abnormal metabolites. One of these may well be responsible for the false-positive tests with the reagent strips and with ferric chloride, but its identity has not been established.

With the present data, one can only speculate on the genetic aspects of this disorder. The patients are sibs. Neither parent has a gross disturbance of histidine metabolism. This suggests that if the anomaly is an inborn error of metabolism, it is transmitted as a recessive. The mother's blood and urinary histidine levels, however, were at the upper limit of normal. Preliminary chromatographic study of the urine of the other members of the family indicates that 5 have a slight increase of histidine in the urine. Four out of these 5 are female. These findings, if confirmed, suggest that a mild disturbance of histidine metabolism may be demonstrable in the female heterozygote.

The relation between the abnormal histidine metabolism and the speech defect in Case 1 is not clear. These children will be watched with great interest. The present findings clearly show that the diagnosis of phenylketonuria should not be made on the results of urinalysis alone.

Summary

The investigation of an assumed case of phenylketonuria brought to light 2 sibs who had consistently raised blood levels of histidine and excreted large amounts of histidine in the urine. The findings suggested that both patients have an inborn error of histidine metabolism.

We are indebted to Dr. J. D. Bailey for referring Case 1 to us.

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MEDICAL PROGRESS

TREATMENT OF CLASSIC HEMOPHILIA: THE USE OF FIBRINOGEN RICH IN FACTOR VIII FOR HEMORRHAGE AND FOR SURGERY*

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CLASSIC hemophilia, comprising about 80 per cent of the heritable coagulation disorders, is characterized by a hemostatic defect largely attributed to deficiency of a clot-promoting principle in the fresh plasma of affected persons. This principle, popularly known as AHG (antihemophilic globulin) or AHF (antihemophilic factor) and officially known as factor VIII, is quantitatively related to thromboplastin generation in vitro and to hemostatic efficiency in vivo. Restoration of plasma factor VIII activity to a clinically useful extent is generally accepted as the rational foundation of the specific therapy of classic hemophilia.

In most cases of classic hemophilia factor VIII deficiency appears to stem from impaired synthesis of this principle, and replacement therapy has been shown to effect a predictable, quantitative increase in plasma factor VIII activity.[1] However, in occasional patients deficient synthesis of factor VIII may be complicated by excessive production of an ill defined inhibitor of factor VIII.[2] Such persons are variably resistant to conventional replacement therapy.

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The results of this study were presented in part at the fourteenth and fifteenth Conferences of the Protein Foundation at Cambridge, Massachusetts, in 1959 and 1960. Transactions of these conferences were published in abstract form in *Vox Sanguinis*.

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Factor VIII replacement therapy of classic hemophilia has largely consisted of the intravenous infusion of fresh normal human plasma. Although sound and useful in this regard, plasma infusion is not free from occasional complications, and at best it is not an efficient source of factor VIII. Specifically, the increment in plasma factor VIII activity that can be effected by fresh plasma therapy is limited by the volume of plasma that can be safely infused. Such increments are sufficient for management of most enclosed soft-tissue hemorrhages but do not ensure adequate replacement in the event of major traumatic or surgical surface wounds.

Factor VIII, like most of the other coagulation factors, has proved to be an elusive principle, and definitive isolation remains to be achieved. However, progress is being made in the concentration of this factor through physicochemical fractionation methods applied to human and animal plasma.

A previous report has described the use of a commercially available fibrinogen fraction rich in factor VIII derived from fraction I of Cohn and his associates,[3] for physiologic studies of clinically stable persons with classic hemophilia.[4] Other currently available fibrinogen preparations that were tested lacked factor VIII activity. These findings will be discussed in detail in a subsequent communication.[5] The purpose of this report is to describe further application of this fraction to the management of a variety of patients with classic hemophilia presenting spontaneous hemorrhage or undergoing surgery.

Material and Methods

Subjects

The subjects of this report are 15 patients who were admitted to the Children's Hospital Medical Center or, in 1 case, to the Peter Bent Brigham Hospital between March, 1959, and December, 1960. All were males, in each of whom a conclusive diagnosis of classic hemophilia was established by an appropriate history and by demonstrable single deficiency of plasma factor VIII activity.

Three members of this group variously exhibited in vitro and in vivo resistance to the clot-promoting effect of all available materials containing this principle. Two of these patients, for whom fresh whole-blood exchange transfusions were performed, will be briefly cited in this report and described more fully in a subsequent report.[6]

Therapeutic Material

The fibrinogen fraction rich in factor VIII* (hereafter referred to as fraction I) administered to these patients was commercially prepared from fresh, normal human plasma by an ethanol-water fractionation process, passed through clarifying and sterilizing filter pads, irradiated with ultraviolet light and dried from the frozen state. The completed product was packaged in units each containing 2 gm. of 85 per cent clottable protein. The factor VIII activity per unit of individual lots tested was the equivalent of 130 to 290 ml. of fresh plasma, mean activity being equivalent to 200 ml. of fresh plasma.[5] The final unit thus contained the factor VIII activity of about 200 ml. of fresh plasma and the total protein content of about 30 ml. of plasma, a sevenfold concentration of factor VIII and protein as compared to native plasma. The material was found to be very stable in the dried state. Each unit was reconstituted with an accompanying 200 ml. of distilled water for injection and administered intravenously through a standard filter. The infusion time per unit varied from ten to thirty minutes, fifteen minutes being modal.

The quantity and frequency of intravenous infusion of the reconstituted fraction was based on the range of plasma factor VIII activity desired. The quantity necessary for a given level of activity in a given patient could be usefully approximated by the following formulation:

$$AHF_1 = AHF_0 + \frac{100\ P}{V + P\dagger}.$$

where AHF_1 = postinfusion plasma factor VIII activity, % normal

AHF_0 = preinfusion plasma factor VIII activity, % normal

P = required fresh plasma, ml. (2 gm. of fraction I = $\lessgtr \pm 200$ ml. fresh plasma depending on lot used)

V = approximate plasma volume, ml. (50 ml. per kilogram of body weight).

Fraction I was administered to patients without evidence of hemorrhage at intervals of twelve to twenty-four hours in most cases. The basis for this timing, demonstrably satisfactory in the majority of cases, was the repeated finding in clinically stable hemophiliac patients not resistant to replacement therapy of a plasma factor VIII half time of ten to fifteen hours.[4]

Methods

Before institution of therapy the following tests were variously utilized for establishing a definitive diagnosis of classic hemophilia:

Whole-blood clotting time in untreated and siliconized glass tubes. Clotting times of venous blood were determined by a modification of the method

*In the estimation of plasma factor VIII activity after infusion of fraction I the volume of fraction I could be ignored in view of its low total protein content (about 1/7 total protein of an equal volume of plasma). Thus, after infusion of fraction I, $AHF_1 = AHF_0 + 100\ P/V$.

†Supplied for investigation by Merck Sharp and Dohme Research Laboratories, Division of Merck and Company, Incorporated.

of Lee and White.[7] After a clean venepuncture with a 19-gauge or 20-gauge, untreated needle, the initial 4.5 ml. of blood was added to 0.5 ml. of 0.1-molar sodium oxalate (for subsequent use as a source of plasma); 10 ml. of blood was next carefully drawn into a fresh plastic syringe,* and 2 ml. of this sample was then introduced into each of 4 clean, dry glass tubes, 13 by 100 mm., 2 of which were untreated and 2 siliconized. (The remaining 2 ml. was transferred to a clean, dry untreated glass tube of the same dimensions and left undisturbed for one hour for measurement of residual serum prothrombin as described below under prothrombin consumption.) All tubes were promptly transferred to a 37°C. water bath. One of the untreated and 1 of the siliconized tubes were then tilted approximately 45° at one-minute intervals until the tubes could be inverted without loss of blood.[7] The same procedure was then followed with the 2 remaining (and previously undisturbed) clotting-time tubes. Final results were expressed as 2 clotting times in untreated glass and 2 clotting times in siliconized glass. (The normal range for whole-blood clotting time in untreated glass is five to fifteen minutes, and that in siliconized glass fifteen to thirty-five minutes.)

One-stage "prothrombin" time. The test of Quick[8] was performed in duplicate on oxalated plasma samples with the use of commercial rabbit brain† as thromboplastin (the normal range is twelve to seventeen seconds).

Plasma prothrombin activity. A one-stage method for assay of prothrombin activity was performed according to Alexander's[9] modification of the Owren[10] method (the normal range is 60 to 120 per cent of normal).

Sixty-minute prothrombin consumption. To 2 ml. of whole blood was added, after one hour of incubation at 37°C., 0.2 ml. of 0.1-molar sodium citrate. Serum was obtained from this sample after centrifugation for two to four minutes at 2000 r.p.m. in room temperature. Prothrombin activity in this serum was determined by the method described above for assay of plasma prothrombin activity. Prothrombin consumption was determined by use of values for plasma and serum activities in the following calculation:

$$\frac{\text{plasma prothrombin (\%)} - \text{serum prothrombin (\%)}}{\text{plasma prothrombin (\%)}} \times 100 = \text{\% prothrombin consumption (normal range, 80 to 100\% consumed).}$$

Plasma factor V activity. A one-stage method for assay of factor V in samples of oxalated or citrated plasma was performed according to the method of Owren,[10] an appropriately aged, normal, oxalated plasma with a Quick time of fifty to seventy seconds being used as substrate (the normal range is 60 to 120 per cent of normal).

Plasma factor VII-X complex activity. A one-stage method for assay of the factor VII-X complex in samples of oxalated or citrated plasma was performed according to the method of Owren,[10] a Seitz-filtered oxalated bovine plasma being used as substrate (the normal range is 60 to 120 per cent of normal).

Plasma partial thromboplastin time. The partial thromboplastin time of oxalated or citrated plasma samples was determined according to the method of Langdell, Wagner and Brinkhous,[11] modified by the use of a 1:10,000 dilution of commercial rabbit brain (Difco) in physiologic saline solution as a partial thromboplastin (the normal range is eighty to one hundred and twenty seconds).

Thromboplastin-generation test. The method of Biggs and Douglas[12] was carried out with the following modifications: a solution of a phospholipid mixture derived from soybeans‡ (25 mg. per 100 ml. of physiologic saline solution) was used as a platelet substitute; imidazole buffer, prepared according to Biggs and Macfarlane,[13] was used for dilution of adsorbed plasma and aged serum samples (the normal range is a minimum substrate clotting time of seven to ten seconds within four minutes).

Plasma fibrinogen. Plasma fibrinogen was measured according to the method of Ratnoff and Menzie[14] (the normal range is 175 to 415 mg. per 100 ml. of plasma).

Bleeding time. Bleeding time was determined by a modification of the method described by Ivy and his associates.[15] Two stabs, about 2.5 cm. apart, were made on the volar surface of the forearm, a blood-pressure cuff having been secured to the same arm and inflated to 40 mm. of mercury. The stabs were made with a Vim sterile-disposable blood lancet.§ The shed blood was blotted at thirty-second intervals with Whatman No. 1 filter paper. The end point was taken as cessation of bleeding from the stab wound exhibiting longer duration of bleeding (the normal range is two to ten minutes).

Assay of plasma factor VIII activity. Factor VIII activity in an unknown sample of oxalated plasma adsorbed with barium sulfate was quantitated by serial thromboplastin-generation tests in which dilutions of unknown and standard plasma were compared. This method was based on the principles

*TOMAC Disposable Syringe, American Hospital Corporation, Evanston, Illinois.

†Available from Difco Laboratories, Detroit, Michigan.

‡In the form of Inositbin, Associated Concentrates, Woodside, Long Island, New York.

§Available from American Cyanamid Company, Surgical Products Division, New York City.

of the factor VIII assay of Biggs, Eveling and Richards.[16] The determined activity of an unknown plasma was expressed as percentage of the normal standard, taken as 100 per cent.

Reagents. Standard plasma was obtained from an adult male donor whose plasma factor VIII activity was modal with respect to plasma activities of more than 10 other normal persons; 9 ml. of whole blood was added to 1 ml. of 0.1-molar sodium oxalate, and the plasma was separated after centrifugation for ten minutes at 5°C. at 1500 r.p.m. The plasma was added to barium sulfate (0.1 gm. of barium sulfate per 1 ml. of plasma), and the mixture shaken in 5°C. for twenty minutes with an automatic test-tube shaker, and centrifuged for fifteen minutes at 5°C. at 3000 r.p.m. The supernatant adsorbed plasma was frozen at −20°C. in 0.2-ml. aliquots sufficient for two or three weeks of ordinary use. These were thawed singly as needed before testing, diluted as described below with imidazole buffer prepared according to Biggs and Macfarlane.[13]

Unknown plasma was processed in the same manner as the standard plasma and was either tested within one hour of adsorption and centrifugation or frozen after adsorption and tested subsequently.

Normal serum, pooled from 3 standard normal donors and aged at room temperature for twelve to twenty-four hours, was frozen in 0.2-ml. aliquots at −20°C. These were thawed singly as desired, diluted 1:20 with imidazole buffer and allowed to stand at room temperature for at least two hours before use in testing.

Citrated plasma from a patient with severe, classic hemophilia was adsorbed with aluminum hydroxide, demonstrably free from clot-inhibiting effects (as described below) and used as a source of factor V. Fresh adsorbed plasma from a standard hemophilic donor was frozen at −20°C. in 0.2-ml. aliquots, thawed singly as needed, diluted 1:20 with imidazole buffer before use in testing and kept in a melting-ice bath throughout use.

The phospholipid mixture derived from soybeans, referred to above (25 mg. per 100 ml. of physiologic saline solution), was used as a platelet substitute. Aliquots of 3 to 5 ml. each were frozen at −20°C., thawed singly as needed and kept at room temperature throughout testing.

An aqueous solution of 0.025-molar calcium chloride was used for recalcification in all stages of testing and was kept at 37°C.

Normal platelet-poor citrated plasma was obtained from the Children's Hospital Medical Center Blood Bank, derived from the preparation of fresh-frozen plasma. This plasma was divided into aliquots of 3 to 5 ml., stored at −20°C., thawed singly as needed and used as substrate in the second stage of the thromboplastin-generation tests.

Test procedure. A freshly thawed 0.2-ml. aliquot of standard adsorbed plasma was diluted 1:5 by the addition of 0.8 ml. of imidazole buffer. With a series of 7 glass tubes, 13 by 100 mm., each containing 0.2 ml. of buffer, consecutive doubling dilutions of the initial 1:5 dilution of the standard plasma were prepared — for example, 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320 and a buffer control. These dilutions were kept in melting ice. Unknown adsorbed plasma was processed at the same time and in the same manner.

A thromboplastin-generating mixture was completed by the addition, to the 0.2 ml. of adsorbed plasma in a given tube, of the following ingredients: 0.2 ml. of diluted, aged normal serum; 0.2 ml. of diluted, adsorbed hemophilic plasma; 0.2 ml. of the soybean-derived phospholipid in solution; and 0.2 ml. of 0.025-molar calcium chloride. A thromboplastin-generation test was completed by the addition, at one-minute intervals, of 0.1 ml. of the incubation mixture followed by 0.1 ml. of substrate plasma, to 0.1 ml. of 0.025-molar calcium chloride. Two stop watches were needed for each test: the first for timing of incubation upon initial addition of calcium chloride (Stage 1), and the second for timing of substrate plasma clot formation upon addition of substrate plasma to the mixture of calcium chloride and the incubation-mixture aliquot (Stage 2). The end point in each test was the minimum substrate clotting time, usually reached within two to five minutes of incubation.

In the manner described above, consecutive thromboplastin-generation tests were performed, standard and unknown adsorbed plasma available in a wide range of dilutions being used. In practice, usually 4 dilutions of the standard (1:40, 1:80, 1:160 and 1:320) and at least 2 dilutions of the unknown were tested in parallel series for comparison. Once testing was actually begun, assay of an unknown plasma (representing 6 to 8 thromboplastin-generation tests) required thirty to sixty minutes for completion.

Calculation of plasma factor VIII activity. During the period of this study, satisfactory reproducibility of minimum substrate clotting times in relation to dilutions of standard plasma was obtained (Table 1). On the basis of this relation a standard curve could be constructed to which dilutions of unknown plasma could be referred for quantitation of factor VIII activity (Fig. 1). The result was calculated from dilutions of known and unknown adsorbed plasma having the same activity — that is,

producing the same minimum substrate clotting time:

$$\frac{\text{reciprocal of unknown plasma dilution}}{\text{reciprocal of standard plasma dilution}} \times 100 = \text{plasma factor VIII activity, \% normal.}$$

The mean factor VIII activity of the unknown plasma was the average of the results calculated from the various dilutions tested. Agreement of results within 20 per cent of the mean was regarded as satisfactory. Plasma factor VIII determinations on more than 20 normal subjects over a given time showed values ranging from 60 to 180 per cent of the standard. However, separation of activities above 30 per cent was inherently unsatisfactory because of disproportionate effect on calculated factor VIII activity of insignificant changes of the minimum substrate clotting time. On the other hand, separation of activities in the particularly critical range of 0 to 30 per cent was quite satisfactory, rendering the assay a useful guide to therapy. (Of interest in these regards was the finding of unmistakably high plasma factor VIII activity — more than 200 per cent of the standard — in 5 randomly tested patients with thrombocytopenia and 1 female tested in the third trimester of a normal pregnancy.)

TABLE 1. *Results of Serial Thromboplastin-Generation Tests.**

DILUTION	MINIMUM SUBSTRATE CLOTTING TIME	INCUBATION TIME
	sec.	*min.*
Standard adsorbed plasma:		
1:5	8 (±1)	2-4
1:10	9 (±1)	2-4
1:20	11 (±1)	2-4
1:40	13 (±1)	2-4
1:80	16 (±2)	2-4
1:160	19 (±2)	2-5
1:320	23 (±3)	3-6
Buffer control	45 (±10)	3-6

*For standard reference curve used in assay of plasma factor VIII activity of unknown adsorbed plasma samples, dilutions of standard plotted against corresponding minimum substrate clotting times on double-logarithmic paper.

Activity of plasma factor VIII inhibitor. Gross assessment of plasma clot-inhibiting activity, assumed to be directed against factor VIII if encountered in a patient known to have classic hemophilia with a normal Quick time, was carried out by mixture of normal and abnormal ingredients in prothrombin-consumption and thromboplastin-generation tests. These tests were adapted as follows from the methods described above.

In the prothrombin consumption test, the addition of 0.1 ml. of fresh, normal oxalated plasma adsorbed with barium sulfate to 2 ml. of freshly shed abnormal blood produced no significant improvement in sixty minutes' prothrombin consumption as compared with a corresponding sample to which 0.1 ml. of physiologic saline solution was added. The application of this technic to freshly shed blood of patients with severe hemophilia without clot-inhibiting activity revealed normal prothrombin consumption in the sample with added normal plasma and, as expected, poor or absent consumption in the sample with added saline solution. This procedure was found to be dependable for assessing activity of factor VIII inhibitor in vitro.

The addition of undiluted abnormal oxalated plasma adsorbed with barium sulfate to a normal thromboplastin-generating mixture, recalcified with 0.05-molar calcium chloride and tested after ten minutes' incubation at 37°C., reduced thromboplastin generation as compared with the effect of controls (saline solution, normal adsorbed plasma and inhibitor-free adsorbed hemophilic plasma) added to similar mixtures, all tested in serial fashion. This test tended to be difficult to reproduce and was never relied upon exclusively.

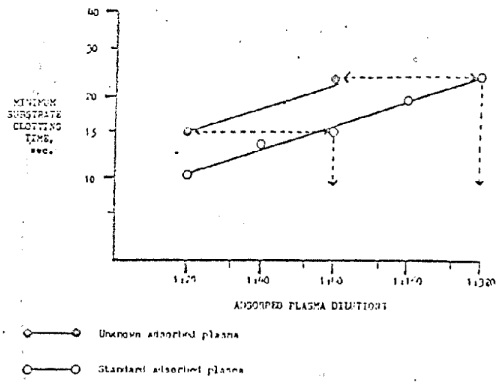

FIGURE 1. *Determination of Plasma Factor VIII Activity in an Unknown Sample by Means of Reference to a Standard Curve Representing 100 per Cent Activity.*

These data are taken from Case 8, on the fifth day after appendectomy; 1:20 and 1:80 dilutions of the unknown sample are equivalent in activity to 1:80 and 1:320 dilutions of the standard respectively. The plasma factor VIII activity of the unknown sample is therefore 25 per cent.

Before initiation of any surgical procedure the patient's responsiveness to replacement therapy was assessed by the effects of infusion of a given quantity of fraction I. A quantitative increase in plasma factor VIII activity was regarded as the most reliable evidence against resistance to replacement therapy (which would, of course, render a major surgical procedure extremely hazardous). In patients being prepared for elective surgery, a twenty-four-hour factor VIII turnover study was carried out after the preliminary infusion of fraction I.

During therapy blood samples for coagulation studies were generally collected once daily and at

a given time in the therapeutic sequence. Plasma factor VIII activity was assayed in all samples, and plasma fibrinogen was measured in most. Other coagulation factors and functions were variably examined as desired.

Peripheral blood examinations were performed as indicated by conventional hematologic methods. In selected cases osmotic fragility of the red blood cells was determined by the method of Dacie,[17] and plasma hemoglobin by the method of Crosby and Furth.[18]

replacement therapy. In these administration of fraction I undoubtedly was causally related to cessation of hematuria in 2 patients (Cases 1 and 2) and supported uneventful surgical aspiration of a hemarthrotic knee (Case 3). Gross clearing of hematuria in both Cases 1 and 2 occurred within six hours of single 4-gm. infusions of fraction I, with concomitant

TABLE 2. *Therapeutic Uses of Fraction I in Classic Hemophilia, March, 1959, to December, 1960.*

CASE NO.	AGE	PLASMA FACTOR VIII ACTIVITY	PRESENTING PROBLEM	SURGICAL TREATMENT	DURATION OF REPLACEMENT THERAPY	RESULT
	yr.	%				
1	8	<1	Hematuria	None	Single dose (4 gm. of fraction I)	Clearing of hematuria within 6 hr. of single dose
2	14	4	Hematuria	None	Single dose (4 gm. of fraction I)	Clearing of hematuria within 6 hr. of single dose
3	2	<1	Hemarthrosis, left knee	Surgical aspiration	2 days (fraction I only)	Uneventful resolution of hemarthrosis
4	15	0 (+ factor VIII inhibitor)	Recurrent surface & soft-tissue hemorrhage	None	Fresh-whole-blood exchange transfusion	Transient rise in plasma factor VIII activity, with diminishing transfusion requirement
5	19	<1 (+ factor VIII inhibitor)	Soft-tissue hemorrhage into face & neck	None	Fresh-whole-blood exchange transfusion	Transient rise in plasma factor VIII activity, with arrest of hemorrhage
6	9	12	Traumatic laceration of chin	None	5 days (plasma) + 7 days (fraction I)	Uneventful healing of wound only on institution of fraction I therapy
7	1	<1	Foreign body in esophagus	Esophagoscopy	7 days (fraction I only)	Uneventful operative & postoperative course
8	13	<1	Appendicitis	Appendectomy	13 days (fraction I only)	Uneventful operative & postoperative course
9	14	6	Appendicitis	Appendectomy	14 days (fraction I only)	Uneventful operative & postoperative course
10a	17	3	Dental abscesses	Drainage; extraction of 2 molars.	10 days (fraction I only)	Uneventful operative & postoperative course
10b			Dental abscesses	Drainage; extraction of 2 molars.	1 day (fresh-frozen plasma) + 9 days (fraction I only)	Brisk hemorrhage immediately after operation, ceasing on institution of fraction I; moderate oozing despite fraction I, 5th–7th postoperative days.
11	12	1	Dental abscess	Drainage; extraction of 1 molar.	11 days (fraction I only)	Transient oozing between 5th & 7th postoperative days
12	<1 (2 days)	2	Hemoperitoneum due to avulsion of splenic pedicle	Splenectomy	Fresh-whole-blood exchange transfusion + 12 days (fraction I)	Diagnosis established after operation; prompt control of hemorrhage on institution of replacement therapy.
13	9	6	Phimosis	Circumcision	4 days (fraction I only) + 6 days (fraction I only)	Bleeding after initial cessation of therapy; uneventful course after reinstitution of therapy.
14	64*	10	Benign rectal polyp	Excision of rectal polyp	7 days (fraction I only) + 9 days (fraction I, fresh whole blood & fresh plasma)	Bleeding after initial cessation of therapy; intermittent bleeding after reinstitution of therapy despite optimal plasma factor VIII activity.
15	15	<1	Complications of portal hypertension	Splenectomy; splenorenal shunt; ligation of esophageal varices.	3 days (fraction I only) + 5 days (fresh whole blood & fresh-frozen plasma)	Development of factor VIII inhibitor on 3d postoperative day; death on 9th postoperative day from complications of hepatic coma.

*Patient at Peter Bent Brigham Hospital, Boston.

RESULTS

The salient clinical features of the subjects of this report, their presenting problems and the results of therapy are summarized in Table 2.

Effects of Fraction I in Management of Renal and Soft-Tissue Hemorrhage (Cases 1 to 5)

Three patients in this group were responsive to elevation of plasma factor VIII activity to the range of 30 to 60 per cent. Both patients had received fresh-frozen plasma before fraction I therapy, without apparent effect on the hematuria. Whether a single infusion of factor VIII concentrate, calculated to produce high levels of plasma factor VIII activity, will prove to suffice in the management of hematuria remains to be seen. Nevertheless, the prompt control of hematuria in these cases strikingly contrasts with previous experience, in which even prolonged replacement therapy with plasma has not been clearly effective, presumably because of insufficient plasma factor VIII activity achieved by such therapy.

Two patients in this group (Cases 4 and 5) were unresponsive to massive replacement therapy utilizing fraction I, fresh-frozen plasma and fresh whole blood. In the course of administered therapy even the whole-blood clotting time in glass, which is reduced to normal limits by less than 5 per cent plasma factor VIII activity, remained grossly prolonged. Coagulation studies showed in both cases potent plasma clot-inhibiting activity affecting thromboplastin generation and prothrombin consumption. These patients received fresh-whole-blood exchange transfusions for intractable soft-tissue hemorrhage, with clinically effective, though temporary, improvement in hemostasis.

Effects of Fraction I in Management of Traumatic Episodes (Cases 6 and 7)

Fraction I was used in the management of a child with mild hemophilia (Case 6) in whom extensive hemorrhage developed into the soft tissue of the chin surrounding an accidentally sustained laceration. Initial replacement therapy with fresh plasma supported resolution of the edema and most of the soft-tissue hemorrhage in the chin. However, the margins of the laceration remained separated by persistent formation of large, black, friable clots within the wound. To ensure adequate factor VIII replacement therapy, fraction I was instituted, and a pressure dressing was applied to the chin after evacuation of all clot formations from the wound. Plasma factor VIII activity was maintained between 15 and 30 per cent during seven days of therapy utilizing 4 gm. of fraction I initially followed by 2 gm. once daily thereafter. Healing of the lesion progressed uneventfully.

An infant with severe hemophilia (Case 7), who had ingested the detached metal tip of a bathinette drainage hose, was treated prophylactically with fraction I before removal of the foreign body by esophagoscopy. The object was successfully removed without complicating hemorrhage, but the procedure was moderately difficult. Accordingly, fraction I was continued for seven days to preclude hemorrhagic complications after instrumentation. Measured plasma factor VIII activity varied between 5 and 30 per cent during therapy, utilizing one infusion of fraction I daily for three days followed by single infusions on the fifth and seventh days.

(To be concluded)

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Acute pericarditis occurred in 3 and probable acute peritonitis in 1 other patients.

No antitumor activity was demonstrated, and the drug is not considered to have any clinical usefulness.

It is emphasized that this represents the first antibiotic and antitumor agent interfering with nucleic acid synthesis that has been demonstrated to produce acute pericarditis. The conjectural implications of this fact regarding possible toxicity of newer drugs being developed is considered.

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MEDICAL PROGRESS

TREATMENT OF CLASSIC HEMOPHILIA: THE USE OF FIBRINOGEN RICH IN FACTOR VIII FOR HEMORRHAGE AND FOR SURGERY (Concluded)*

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Effects of Fraction I in Surgical Intervention (Cases 8-15)

As applied to the supportive management of patients with classic hemophilia undergoing various emergency and elective surgical procedures, fraction I therapy was associated with a spectrum of effects.

Fraction I therapy instituted before and adequately maintained after operation. In 2 cases of appendectomy (Cases 8 and 9) and 2 of dental extractions (Cases 10a and 11), the exclusive use of fraction I for replacement therapy supported uneventful operative and postoperative phases. These surgical procedures progressed within clearly normal limits with 1 exception (Case 11), in which elective extraction of an abscessed molar in a patient with mild hemophilia was followed by minimal oozing transiently between the fifth and seventh postoperative days.

The absence of significant hemorrhagic complications in the cases referred to above was attributed to three major factors obtained in all: meticulous surgical hemostasis; a plasma factor VIII activity of

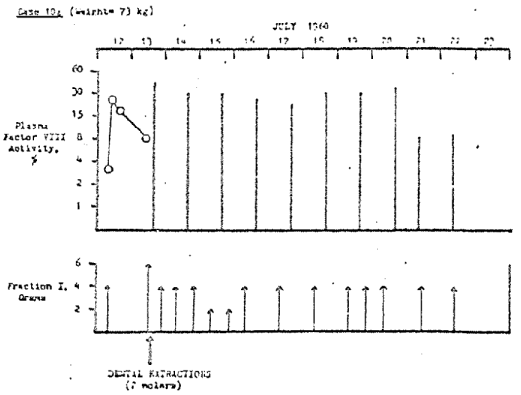

FIGURE 2. *Record of Fraction I Administered and Resulting Plasma Factor VIII Activity in Case 10a, Uneventfully Undergoing Dental Extractions.*

30 to 60 per cent established at the time of surgery; and maintenance of plasma factor VIII activity of 15 to 30 per cent for a minimum of ten days after operation. The relation of dosage, timing and dura-

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The results of this study were presented in part at the fourteenth and fifteenth Conferences of the Protein Foundation at Cambridge, Massachusetts, in 1959 and 1960. Transactions of these conferences were published in abstract form in *Vox Sanguinis*.

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tion of fraction I therapy to resulting factor VIII activity is shown in a representative case (Fig. 2).

Fraction I therapy instituted after operation and adequately maintained. That fraction I was causally related to efficient hemostasis in the cases cited above was suggested in 1 case (Case 10b) in which significant operative bleeding complicated extraction of 2 molars supported by preoperative administration of fresh plasma alone. Previous extractions of a comparable nature with the use of fraction I (Case 10a) had been entirely uneventful. The administration of 1200 ml. of fresh-frozen plasma, designed to raise plasma factor VIII activity to about 25 per cent in this patient (73-kg. body weight), raised the level of activity only to 15 per cent. Brisk bleeding developed during surgery and failed to respond satisfactorily to local pressure and fresh-frozen plasma continued in a dose of 600 to 700 ml. every eight hours. Because of continued oozing up to twenty-four hours after surgery, fraction I was instituted in place of plasma, with prompt control of hemorrhage. However, despite continued replacement therapy, moderate recurrent oozing from the extraction sites was noted between the fifth and seventh postoperative days (Case 11).

Excessive bleeding complicated splenectomy in a two-day-old infant with unexplained avulsion of the splenic pedicle (Case 12). In this case the diagnosis of classic hemophilia, unsuspected because of a negative family history, was established after operation when abnormal hemorrhage was apparent. Hemorrhage through the surgical wound occurred but was controlled with an exchange transfusion of fresh whole blood, and hemostasis was satisfactorily supported by infusions of fraction I continued for a total of twelve days.

Fraction I therapy instituted before and inadequately maintained after operation. Patients with mild hemophilia undergoing minor operations (Cases 13 and 14) showed no complications during initial courses of therapy of four and seven days respectively. However, in both patients hemorrhagic complications developed within forty-eight hours of termination of these courses of therapy.

Postcircumcision bleeding was promptly controlled upon reinstitution of fraction I therapy, immediately followed by ligation of a spurting frenular artery and application to the wound of a pressure dressing with a vaseline base. Uneventful healing ensued during maintenance of fraction I therapy for an additional six days with a plasma factor VIII activity of 15 to 30 per cent during this time.

Postpolypectomy bleeding was not controlled with doses of fraction I sufficient to maintain plasma factor VIII activity in an optimal range. Intermittent rectal hemorrhage, beginning on the ninth and finally terminating on the eighteenth postoperative day, likewise was not clearly influenced by administration to the patient of fresh blood, fresh plasma and prednisone. No evidence for complicating platelet, vascular or unforeseen coagulation defect was obtained during this episode of delayed hemorrhage. Because the bleeding was not massive and the outcome of local hemostatic therapy was regarded as uncertain at best, local measures were deferred. The course of this patient is shown in Figure 3.

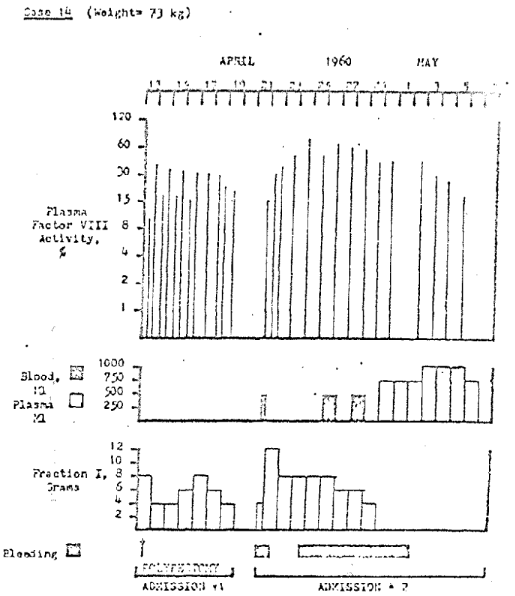

FIGURE 3. *Record of Fraction I Administered and Resulting Plasma Factor VIII Activity in Case 14, Undergoing Uneventful Excision of a Rectal Polyp but Suffering Hemorrhage after Termination of the Initial Period of Replacement Therapy.*

Fraction I therapy instituted before operation and complicated by postoperative factor VIII inhibitor state. Resistance to the effects of factor VIII added in vitro and in vivo developed in the postoperative period of 1 patient (Case 15). He uneventfully underwent extensive surgical procedures, including splenectomy, splenorenal shunt, ligation of esophageal varices and liver biopsy. These procedures were performed for relief of portal hypertension complicated by bleeding esophageal varices, splenomegaly and pancytopenia in a fifteen-year-old boy with a well documented episode of hepatitis at eight years of age. After hepatitis (though not necessarily because of it) resistance to replacement therapy developed. A factor VIII inhibitor state was thoroughly documented three months before this admission. However, on admission to the hospital for treatment of massive hematemesis three days before the operation described above, there was — for unknown reasons — no evidence of the inhibitor state. Predictable response to therapy persisted through the surgical procedure and the third postoperative day. At the end of this

period, despite massive replacement therapy, plasma factor VIII activity rapidly disappeared, and a progressive downward course ensued, terminating in death from complications of hepatic coma on the ninth postoperative day.

Of note in this patient was the development of hemoglobinemia and hemoglobinuria on the third postoperative day in the course of massive fraction I therapy. These phenomena, associated with a plasma fibrinogen of 1200 mg. per 100 ml. of plasma and marked agglutinability of the red blood cells, cleared spontaneously and without apparent residual after discontinuation of fraction I therapy and consequent return of fibrinogen to physiologic levels. During the

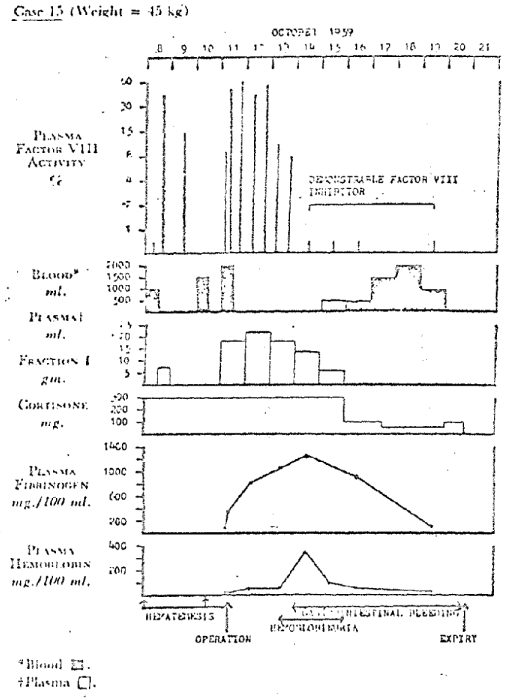

FIGURE 4. *Record of the Course of Case 15, Successfully Undergoing Extensive Surgery for Complications of Portal Hypertension but Manifesting a Factor VIII Inhibitor after Operation and Succumbing to Hepatic Coma.*

phase of hyperfibrinogenemia clumping of the red cells was so marked that all typing procedures were obscured despite liberal washings with physiologic saline solution.

Pertinent data in this case are shown in Figure 4.

General Observations on Effects of Fraction I Therapy

Coagulation tests reflecting factor VIII activity. As a guide to replacement therapy reliance was placed on specific assay of plasma factor VIII activity rather than on functional tests known to reflect such activity. Tests reflecting factor VIII activity include the whole-blood clotting time in untreated and siliconized glass tubes, prothrombin consumption, plasma recalcification and partial thromboplastin times, and the thromboplastin-generation test.

Random observations in the course of therapy, in accord with the data of Biggs and Macfarlane,[18,19] suggested insensitivity of the whole-blood clotting time in untreated glass tubes to more than 5 per cent plasma factor VIII activity, of prothrombin consumption to more than 10 per cent activity and of the unmodified thromboplastin-generation test to more than 20 per cent activity. These tests were therefore not regarded as sufficiently sensitive for guiding rational management of critical surgical episodes. Furthermore, clotting time and even prothrombin-consumption measurements are recognized as being unduly affected by technical variables. In small children undergoing prolonged intravenous therapy blood samples for such measurements could not be consistently obtained.

Limited observations on the plasma recalcification time and partial thromboplastin time suggested broad correlation of results of these tests with plasma factor VIII activity. However, the limits of their usefulness in guiding fraction I therapy were not established. Efforts to utilize these tests for factor VIII assay, as described by others,[11,20] were unsuccessful.

Curiously enough, the whole-blood clotting time in siliconized glass tubes, although shortened after infusion of fraction I, was not clearly reduced to normal limits despite nearly normal plasma factor VIII activity. However, in vitro additions to freshly shed hemophilic blood of fraction I solution (with calcium chloride added to offset the citrate content of fraction I), of fresh plasma, of aged plasma or of serum were all equally effective in normalizing the clotting time in siliconized tubes.[21] These phenomena warrant further elucidation, but from a practical point of view the incomplete correction of hemophilic whole-blood clotting time in siliconized tubes has not appeared to be associated with a significant hemostatic defect in vivo.

The results of whole-blood clotting times in untreated and siliconized glass tubes and plasma partial thromboplastin times as compared with plasma factor VIII activity are shown in Figure 5. These data were obtained in connection with fraction I therapy of a patient (Case 8) uneventfully undergoing appendectomy.

Hyperfibrinogenemia. In all patients undergoing continuous replacement therapy with fraction I for a week or more, fibrinogen levels in the range of 1000 to 1200 mg. per 100 ml. of plasma were regularly observed. Rouleau formation and rapid sedimentation of the red blood cells were noted in connection with fibrinogen levels above 500 mg. per 100 ml. of plasma.

Except for 2 patients (Cases 8 and 15) hyper-

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fibrinogenemia was uneventfully tolerated and specifically without evidence of hemolytic phenomena. In the course of fraction I therapy, Case 8 showed a significant fall in levels of hemoglobin and hematocrit,

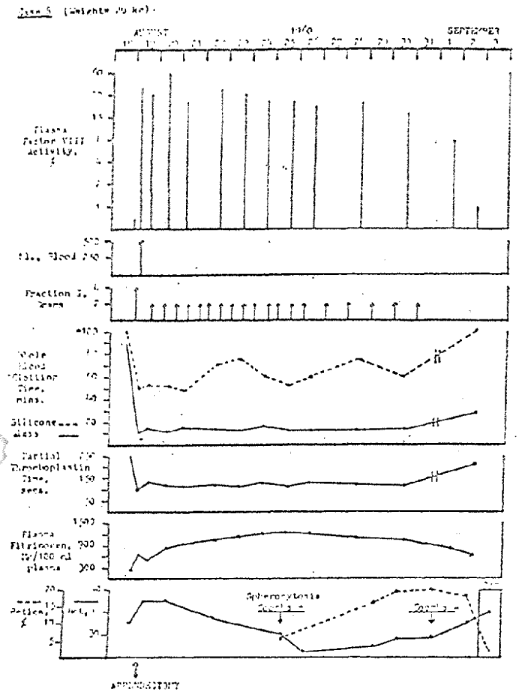

FIGURE 5. *Record of the Course of Case 8, Uneventfully Undergoing Appendectomy, in Whom Multiple Parameters Were Followed.*

Concomitant with plasma factor VIII activity between 15 and 60 per cent the patient showed normal whole-blood clotting times in glass and marked improvement in the partial thromboplastin time. However, the whole-blood clotting time in siliconized tubes, although shortened, showed irregular fluctuations and was at no time clearly within normal limits. (The partial thromboplastin-time values represent the mean of duplicate determinations; the whole-blood clotting times were not done in duplicate.) Concomitant with hyperfibrinogenemia, a significant fall in hemoglobin developed in association with reticulocytosis, spherocytosis, a false-positive Coombs test and increased osmotic fragility not associated with blood loss except for 10-ml. samples of venous blood obtained in connection with coagulation studies. The indications of hemolytic phenomena cleared spontaneously with the return of fibrinogen to physiologic levels.

reticulocytosis, minimal hyperbilirubinemia and development of spherocytosis, with increased osmotic fragility of the red blood cells (Fig. 5). There was no evidence of concomitant blood loss. These phenomena cleared spontaneously coincident with return of fibrinogen to physiologic levels. As noted previously, transient hemoglobinemia and hemoglobinuria occurred in the postoperative course of Case 15, who received large doses of fraction I in connection with an extensive surgical procedure, including splenectomy. In this patient spherocytosis was not observed, and studies of osmotic fragility were not carried out. In both these patients false-positive Coombs tests were noted during the period in which fibrinogen levels were more than 1000 mg. per 100 ml. of plasma.

Infusion reactions. Fraction I infusions were clinically well tolerated in all patients except Case 12. On two occasions the infusion into this 3.8-kg. (8½-pound) newborn infant of 40 to 50 ml. of 2 per cent fraction I in less than ten minutes was associated with apneic episodes from which he recovered without evident sequelae. On other occasions infusion times of at least thirty minutes each were uneventful. These episodes were unexplained but suggest the desirability of slower infusion times in small infants receiving fraction I.

Hepatitis. Mild and transient hepatitis was observed in 1 patient (Case 14), beginning thirty-five days after initiation of fraction I therapy. Excluding Case 15, the remainder of patients have so far shown no clinical evidence of hepatitis.

Discussion

Although salient gaps remain in current knowledge of classic hemophilia and factor VIII, important progress has been achieved in recent years by the development of methods for measuring factor VIII activity and for concentrating this factor from human and animal plasma. The thromboplastin-generation test of Biggs and Douglas,[12] the prothrombin-utilization technic of Graham and his associates[22] and the partial thromboplastin test of Langdell, Wagner and Brinkhous[11] have provided bases for quantitative assessment of plasma factor VIII activity. Such quantitation has, in turn, afforded a more rational approach to the clinical problems of hemophilia and has facilitated evaluation of plasma-fractionation products.

The application of factor VIII quantitation to plasma fractionation and replacement therapy of persons with classic hemophilia is illustrated in the important work of the following groups: Blombäck and Nilsson,[20] using a human plasma fraction (fraction 1-O)[23] to which the fraction used in this study is largely analogous; Kekwick and Wolf,[24] using a fraction prepared from human plasma by the ether-water fractionation process of Kekwick, Mackay and Record[25]; and Macfarlane et al.,[26] using bovine and porcine plasma fractions prepared by Bidwell.[27]

From quantitative studies of the relation of plasma factor VIII activity to hemostasis certain clinically useful generalizations have evolved. These are largely described by Biggs and Macfarlane[13,28] and may be summarized as follows: normally, plasma factor VIII activity ranges from 50 to 200 per cent of a mean of 100 per cent; persons severely affected with classic hemophilia usually show less than 1 per cent activity; persons with 1 to 5 per cent factor VIII activity are

moderately to mildly affected; persons with more than 5 per cent activity are typically mildly affected and require sensitive testing methods for accurate diagnosis; most patients with hemophilia respond to replacement therapy in a rather predictable fashion, but a small number, even when clinically stable, exhibit definite resistance to replacement therapy; in persons not resistant to therapy, disappearance of plasma factor VIII after replacement is exponential, various half-time estimates ranging largely from four to twelve hours (depending on multiple poorly correlated factors including extravascular diffusion, clinical stability and individual variations in the response of patients to replacement therapy); therapy with fresh plasma in a total daily dose as usually employed of 20 to 30 ml. per kilogram of body weight ordinarily ensures plasma factor VIII activity of 5 to 20 per cent and is effective for management of most hemorrhagic episodes occurring in classic hemophilia; optimal supportive management of major traumatic lesions and surgical intervention requires, at least initially, nearly normal plasma factor VIII activity — that is, more than 30 per cent activity regardless of the basal deficiency of the patient; factor VIII concentrates are generally required to achieve plasma factor VIII activity of more than 30 per cent and should be employed in any case of major surgical intervention — never lightly undertaken and hazardous if inadequately supported; and maintenance of replacement therapy after operation is required until disrupted vascular channels are sufficiently repaired to tolerate withdrawal of added factor VIII.

The foregoing generalizations serve as a useful background for scrutiny of the results of this study. For the most part, the generalizations are supported by the results.

Regarding the relation of basal plasma factor VIII activity to general clinical status, the patients with severe hemophilia had virtually no measurable activity while those who were mildly affected had from 3 to 12 per cent activity. In patients not resistant to replacement therapy fraction I produced expected quantitative increase in plasma factor VIII activity, reversed primary hemorrhage and — most significantly — supported uneventful surgical intervention. In the 2 patients (Cases 4 and 5) known to be resistant to replacement therapy, no measurable plasma factor VIII activity could be demonstrated after conventional infusion of fresh whole blood, fresh plasma or fraction I.

In contrast to the finding of less than 30 per cent plasma factor VIII activity in patients with hemophilia even while they were receiving optimal doses of fresh-frozen plasma (such as Case 10b), activities of 30 to 60 per cent were regularly observed in patients treated with fraction I. The principal limitation of fresh plasma as a form of replacement therapy is its inefficiency in terms of the ratio of factor VIII to protein. Although plasma factor VIII activity can be safely raised to normal levels with appropriately concentrated material, such increments cannot be achieved by fresh whole blood or plasma short of exchange-transfusion therapy. Blood and plasma alone have been used successfully for the support of surgery in classic hemophilia.[19,28] However, in most of these cases the operative and postoperative phases have been unpredictable and marked by prolonged hemorrhagic complications. Thus, although unmistakably useful for replacement therapy of most hemorrhagic episodes in classic hemophilia, fresh blood and plasma cannot be depended upon for optimal support of major surgical intervention.

In this study surgical intervention supported by fraction I was uniformly uneventful. Effective hemostasis was attributed to meticulous surgical hemostasis and to 30 to 60 per cent plasma factor VIII activity during surgery in all cases. In addition to optimal hemostasis at the time of surgery, there was evident need for adequate maintenance of therapy after operation. No significant hemorrhage developed in any case in which optimal preoperative replacement therapy was coupled with maintenance of plasma factor VIII activity between 15 and 30 per cent for ten to fourteen days after operation.

However, as described above, in 2 patients (Cases 13 and 14) hemorrhage developed within forty-eight hours after premature discontinuation of replacement therapy. Postcircumcision bleeding in a nine-year-old boy with mild hemophilia (Case 13) was promptly controlled upon reinstitution of fraction I combined with local surgical hemostasis. On the other hand, hemorrhage that followed excision of a rectal polyp in a sixty-four-year-old patient with mild hemophilia (Case 14) was not effectively controlled despite satisfactory plasma factor VIII levels with the use of fraction I variously combined with fresh blood and plasma.

In other circumstances it has been observed[12] that certain surface wounds, notably oral [illegible] troublesome intermittent hemorrhage despite plasma factor VIII activity of 30 per cent or more. Such hemorrhage has usually been delayed in onset by hours or even days after occurrence of a wound for which immediate replacement therapy was not carried out or after cessation of therapy of an inadequately healed wound. Surface lesions exhibiting intermittent hemorrhage of this sort have tended to be diffuse granulating wounds, not amenable to effective local treatment. In general, these lesions have been conservatively managed, with an emphasis on optimal replacement therapy. Despite an occasionally troublesome course, healing has occurred within one to three weeks. It has appeared that the development of troublesome delayed hemorrhage from surface wounds can largely be prevented by adequate maintenance as well as early initiation of replacement therapy, combined with appropriate local hemostasis. These observations have particular implications for

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the management of surgical intervention in classic hemophilia. The mechanism of persistent hemorrhage despite optimal plasma factor VIII activity is not clear. However, it seems reasonable to believe that local factors affecting certain wounds may be largely contributory. These factors, underscored by Macfarlane and his associates,[26] include infection, trauma, insufficient immobilization and disruption of large vessels. It should be emphasized that inadequacy of replacement therapy in such cases is distinct from resistance caused by development of inhibition of plasma factor VIII activity. In the former appropriate increments in plasma factor VIII activity are observed during replacement therapy; in the latter, such increments are not observed, and indeed no increment may be observed at all.

Development of resistance to replacement therapy due to factor VIII inhibition was observed only in the postoperative course of 1 patient in this study (Case 15). However, this patient's resistance to therapy may have been related more to his pre-existing resistant state than to the effect of treatment with fraction I. The mechanism of development of factor VIII resistance is not known. Although the clot-inhibiting fraction observed in these cases has been widely regarded as immune, recent studies by Biggs and Bidwell[2] do not support this hypothesis. Factor VIII concentrates may aggravate a latent or established resistant state but one should not necessarily expect such concentrates to precipitate factor VIII resistance in all cases.

In the course of continuing application of factor VIII concentrates to replacement therapy of classic hemophilia, the concept of this disease as a simple deficiency of factor VIII will be increasingly tested. It is evident that certain aspects of hemophilia are not explained by this concept. These aspects include the existence of factor VIII inhibitor states, the cyclic character of the hemorrhagic tendency in classic hemophilia that cannot be related to fluctuations in plasma factor VIII activity and the unmistakable benefits of appropriate local hemostatic measures.

Nevertheless, the major role of plasma factor VIII activity in hemostasis is clearly established, and quantitative principles of replacement therapy can be usefully applied to the rational management of most hemorrhagic episodes in classic hemophilia. Significantly, the use of fresh plasma in this regard is now augmented with increasingly available factor VIII concentrates that are capable of supporting safely even major surgical intervention.

Summary and Conclusions

A fibrinogen fraction from normal human plasma rich in factor VIII, derived from Cohn's fraction I, was used for replacement therapy of 15 patients with classic hemophilia presenting a variety of hemorrhagic and surgical conditions.

In patients not resistant to replacement therapy the fraction produced the expected increase in recipient plasma factor VIII activity as determined by an assay based on the thromboplastin-generation test. Increase in plasma factor VIII activity correlated with improved hemostatic efficiency as judged by reversal of primary hemorrhagic states and effective support of major surgical intervention. Uneventful surgical intervention appeared to require meticulous local hemostasis and plasma factor VIII activity of 30 to 60 per cent initially and maintenance of plasma activity of 15 to 30 per cent after operation for a minimum of ten days. In a single case, despite optimal plasma factor VIII activity, the fraction was of limited usefulness in the management of intermittent hemorrhage complicating termination of replacement therapy on the seventh postoperative day.

The fraction was ineffective in the management of persons exhibiting in vitro and in vivo resistance to the effects of added factor VIII. Development of resistance to replacement therapy after the administration of fraction I was observed in a single case in a patient with severe hepatitis and liver failure; a pre-existing resistant state, curiously disappearing before a major surgical procedure that was uneventfully completed and included splenectomy and a splenorenal shunt, returned on the third postoperative day.

Marked clumping of the red blood cells was associated with hyperfibrinogenemia developing in all patients undergoing protracted replacement therapy. In 2 patients hemolytic phenomena concomitant with fibrinogen levels of more than 1000 mg. per 100 ml. of plasma were observed. These phenomena cleared without apparent residual after return of fibrinogen to physiologic levels.

Mild and transient hepatitis developed in 1 patient thirty-five days after initiation of fraction I therapy. However, during the hospital course he had also received infusions of fresh whole blood and fresh plasma.

These studies suggest the usefulness of a fraction I rich in factor VIII for replacement therapy of classic hemophilia uncomplicated by factor VIII inhibitor states.

As applied to supportive management of major surgical intervention, this fraction appears to be superior to fresh plasma by virtue of the greater plasma factor VIII activity that can be achieved.

We are indebted to Dr. S. Clyde Strickland and his associates at Merck Sharp and Dohme Research Laboratories, Division of Merck and Company, Incorporated, for the fibrinogen rich in factor VIII used in these studies, to Dr. Frank H. Gardner for permission to include his patient (Case 14) in these studies, to Dr. Herbert S. Strauss, Miss Barbara B. Steele, Miss Angelina Di Francesco and Miss Dorothy Hearne for assistance in these studies, to many members of the surgical, orthopedic, dental and medical house staffs of the Children's Hospital Medical Center for co-operation in the clinical management of the patients included in these studies and to Dr. Charles A. Janeway, Dr. James L.

Tullis, Dr. Robert B. Pennell and Mr. Marshall Melin for their encouragement and advice.

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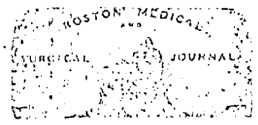
MEDICAL INTELLIGENCE



OBSTRUCTIVE UROPATHY COMPLICATING ANTICOAGULANT THERAPY

WILLIAM S. KADEN, M.D.,* AND
ELI A. FRIEDMAN, M.D.†

BOSTON

THE varied hemorrhagic complications resulting from the increasing use of anticoagulant drugs are under current study.[1-8] Acute medical and surgical diseases may be mimicked by the presence of occult hematoma. In the case presented below, the recognition of a perivesical hematoma as the cause of acute urinary suppression allowed the prompt relief of obstruction and recovery of the patient.

Case Report

A 72-year-old widowed, childless woman (P.B.B.H. 4R478) was admitted to the Peter Bent Brigham Hospital for the 1st time, on October 18, 1960, because of shortness of breath of about 4 months' duration. There had been associated exertional dyspnea, orthopnea and ankle edema. During the year before admission she had experienced oppressive, precordial pains that radiated into the left arm and lasted less than 5 minutes. On the evening of admission she complained of continuous dyspnea and increasing chest discomfort.

The past history and review of the systems were noncontributory.

Physical examination showed a co-operative, intelligent, dyspneic woman. The neck veins, which filled from below, were distended at 60°. There was 4 + 4 pitting edema of the legs, but no petechiae, cyanosis or icterus. The chest was dull to percussion at both lung bases posteriorly and exhibited moist, inspiratory rales to the inferior scapular margins bilaterally. The heart was enlarged, the border extending to the anterior axillary line, with a heaving precordial impulse and a rapid regular rhythm. No murmurs, friction rubs or other adventitious sounds were heard. The abdomen was entirely normal. Neurologic examination was remarkable for a left extensor toe response.

The temperature was 99°F., the radial pulse 100, and the respirations 36. The blood pressure was 140/100.

Pertinent laboratory data included a hematocrit of 46 per cent, a white-cell count of 11,500, with a shift to early granulocytic forms, a urine pH of 6.0, a specific gravity of 1.020, with a trace test for protein and a negative test for sugar; no cells or casts were seen on microscopical examination of the sediment. The following chemical determinations were within normal limits: blood urea nitrogen, fasting blood sugar, sodium, potassium, chloride, carbon dioxide combining power, bilirubin, serum glutamic oxalacetic transaminase and lactic dehydrogenase. An electrocardiogram showed atrial tachycardia and left-bundle-branch block.

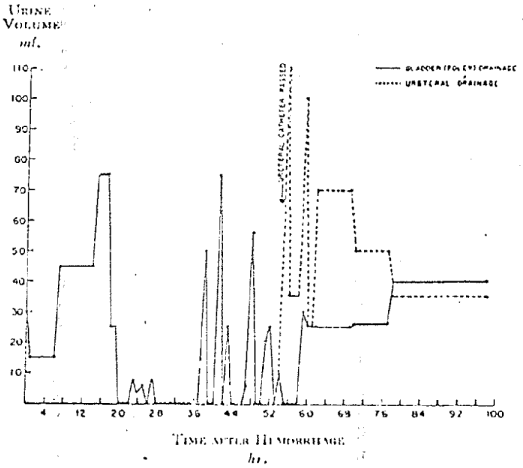


FIGURE 1. *Fluctuating Urinary Outputs Characteristic of Intermittent Obstruction.*

The passage of the ureteral catheter fifty-four hours after hemorrhage resulted in increased and sustained urinary output.

It was initially thought that the congestive heart failure was etiologically related to a pulmonary embolism or myocardial infarction. Accordingly, anticoagulation therapy with warfarin sodium and heparin was begun against a baseline prothrombin time (Quick method) of 55 per cent. On October 21 and 22, 15 mg. of warfarin was given by mouth, with subsequent prothrombin times of 15 per cent on October 22, 15 per cent on October 23 and less than 5 per cent on October 24. Because of the sensitivity to relatively low doses of warfarin, 5 mg. of vitamin K_1 oxide was given, and it was decided to discontinue warfarin therapy and to control the hazards of withdrawal with heparin in a dose of 60 mg. by deep subcutaneous injection every 6 hours. The prothrombin time remained depressed to 7 per cent on October 25 and less than 5 per cent on the following day.

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Antihemophilic Factor VIII in Hemophilia

Use of Concentrates to Permit Major Surgery

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Seven surgical procedures were successfully performed on five patients suffering from classic hemophilia. Hemostasis was maintained by the use of three factor VIII concentrates: cryoprecipitate, American National Red Cross high-purity concentrate, and a lyophilized glycine-precipitated fraction. Significant hemorrhage occurred twice, in each instance when the patient's factor VIII levels fell below 20% of normal. The availability of satisfactory concentrates of factor VIII permits consideration of elective surgical procedures in the hemophiliac. It should be emphasized, however, that such operations should be undertaken only if the indications are unequivocal and the necessary clinical and laboratory facilities are available.

Surgical operations in the hemophiliac have always been serious problems requiring close cooperation by a clinician experienced in the treatment of hemophilia, an expert surgeon, a laboratory able to assay coagulation factors accurately, and a blood bank capable of supplying adequate amounts of blood and appropriate blood components. The concentration of factor VIII (antihemophilic factor or AHF) in normal plasma is inadequate to produce effective hemostasis in the hemophiliac during major surgical procedures and the administration of large volumes of plasma may overload the circulation.

With the advent of factor VIII concentrates, high levels of factor VIII can be maintained and major operations can be performed. Our experience with seven such procedures in five patients with classic hemophilia is described. Their bleeding diathesis was generally well controlled by the use of three factor VIII concentrates: cryoprecipitate, a lyophilized glycine-precipitated fraction, and American National Red Cross (ANRC) high-purity concentrate. Various other factor VIII concentrates will be reviewed.

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Report of Cases

CASE 1.—A 19-year-old boy with documented classic hemophilia had a history of severe bleeding since infancy, including easy bruising, bleeding from the frenulum of the tongue, hemarthrosis, and Volkmann's contractures. After an automobile accident, he had brief amnesia without loss of consciousness. In the ensuing ten days, severe frontal headaches on the right side and then persistent nausea and vomiting developed. During this period he received 5 units (about 250 ml each) of fresh-frozen plasma and 2 units (about 500 ml each) of fresh whole blood without improvement in his condition. He was transferred to the Mayo Clinic where, within a few hours, he had five grand mal seizures. Laboratory findings confirmed the diagnosis of classic hemophilia with a factor VIII level of 1.2% of normal, normal platelet counts, and normal bleeding times.

CASE 2.—A 45-year-old man was known to have had hemophilia since age 5 years. He had had epistaxis as a child and numerous episodes of hemarthrosis and hematuria during the past 40 years. Four years previous to admission the patient had suffered a comminuted fracture of the proximal part of the left femur for which he was treated with traction and fresh-frozen plasma. He subsequently was confined to crutches and a wheelchair because of nonunion of the left femur. In March 1969, he was admitted to the Mayo Clinic for surgical stabilization of the left femur. His plasmatic factor VIII level was found to be 4% of normal. Platelet counts, bleeding times, and platelet adhesiveness were normal.

CASE 3.—A 25-year-old man with classic hemophilia had been seen at the Mayo Clinic on numerous occasions for bleeding problems. His rather typical past history was that of epistaxis, hemarthrosis, and hematuria occurring periodically. In 1961 he suffered a traumatic fracture of the lower part of the right femur and subsequently had surgical treatment elsewhere for nonunion. Lack of success at that time prompted another operation in 1963. Hemarthrosis of the right knee occurred repeatedly thereafter and resulted in ankylosis of the joint. The patient did well until August 1968, when he was again seen at the Mayo Clinic because of blood in the stool. He was hospitalized and given fresh-frozen plasma, and the bleeding stopped. Results of complete examination of the bowel were normal. In October, he again noted blood in the stool and was hospitalized and given fresh-frozen plasma and cryoprecipitate. Bleeding eventually ceased and complete examination of the gastrointestinal tract revealed no lesions in the bowel. Finally, in December, he was hospitalized for the third time because of gastrointestinal bleeding. X-ray films disclosed questionable granulomatous colitis involving the right side of the colon and terminal part of the ileum or questionable submucosal hemorrhage involving the colon. The patient's factor VIII level was less than 1% of normal. Platelet counts and bleeding times were normal.

CASE 4.—A 10-year-old boy was referred to the Mayo

Clinic for evaluation and possible surgical correction of an equinovarus deformity of the right foot present since age 2½ years, due to a crush injury of the right foot and ankle and ensuing ankylosis of the ankle. Considerable shortening of the Achilles tendon prevented dorsiflexion of the foot. In addition, he had a history of hemarthrosis involving other joints, epistaxis, and ecchymosis. In May 1968, he was hospitalized for corrective surgery. His factor VIII level was less than 1% of normal; platelet counts, bleeding times, and platelet adhesiveness were normal.

CASE 5.—A 22-year-old man was known to have had classic hemophilia since early childhood. He had a history of epistaxis, hemarthrosis, and ecchymosis. He had been seen at the Mayo Clinic numerous times for hemarthrosis involving several joints. In 1959, he had broken a femur and was treated nonsurgically, with plasma as the source of factor VIII.[1] For a number of years he had frequent recurrent episodes of bleeding into the left knee. Degenerative changes resulting in severe pain and hemarthroses, and, eventually, contractures severely limited ambulation and required the wearing of a brace on the left leg and intermittent use of a wheelchair. Repeated studies over the years showed a plasmatic factor VIII level of less than 1% of normal and normal platelet counts, bleeding times, and platelet adhesiveness.

Methods

Factor VIII was assayed by a one-stage kaolin-activated partial thromboplastin time method.[2,3] The samples were usually assayed immediately after venipuncture although an occasional nighttime sample was stored at −20 C after the plasma had been separated by centrifugation. Factor VIII level was expressed as a percent of our reference standard which in turn has been standardized against a pool of 59 normals. Factor VIII in the concentrates and in plasma is expressed in units, 1 unit representing the factor VIII activity of 1 ml of the reference standard plasma. In order to predict the rise in factor VIII level after infusion of a factor VIII concentrate, the patient's plasma volume was assumed to be 40 ml/kg of body weight unless the hematocrit value was significantly reduced.

Factor VIII Concentrates

Cryoprecipitate was prepared by the method of Pool and Shannon.[4] In essence, fresh plasma is rapidly frozen (−50 C to −70 C) and then thawed slowly at refrigerator temperature (+4 C). The fibrinogen, with factor VIII absorbed, remains out of solution and is recovered by centrifugation. After removal of the plasma, the fibrinogen-factor VIII precipitate is frozen (−30 C) and subsequently thawed at 37 C for infusion into the patient. Factor VIII recovery averaged about 40% of that in the original plasma, or about 80 units of factor VIII from 200 ml of plasma.

Lyophilized Glycine-Precipitated Fraction.—This substance is prepared commercially by the method of Wagner and colleagues.[5,6] Fresh plasma is freed of prothrombin and other factors by aluminum hydroxide adsorption and the concentration of fibrinogen is reduced by the use of a derivative of fuller's earth (Florigel). The residual fibrinogen plus factor VIII is precipitated by the addition of glycine. The lyophilized precipitate, stable at 4 C, is dissolved in 0.9% sodium chloride solution at 37 C for administration to the patient.

American National Red Cross "High-Purity AHF"[7].—This consists of a polyethylene-glycol precipitate of plasmatic factor VIII and, because of virtual absence of fibrinogen, is very easy to reconstitute from the lyophilized state. This preparation has not yet received approval of the federal Food and Drug Administration for general use.

Seven Operations on Five Hemophiliacs

The 19-year-old boy (case 1) with a head injury received an infusion containing 500 units of factor VIII (cryoprecipiatate). A retrograde arteriogram made via the right brachial artery demonstrated a probable subdural hematoma. After another infusion of 400 units of factor VIII (cryoprecipitate), four burr holes were drilled in the right side of the skull and 30 ml of blood was evacuated. There was no excessive bleeding.

The day after craniotomy the patient was up and about and his headache was gone. Neurologic findings were normal. Over the next 17 days, 6,650 units of factor VIII (cryoprecipitate) were given at the rate of 250 to 500 units a day. Since each bag contained only 15 to 20 ml of cryoprecipitate, there was no circulatory overload and no untoward reactions occurred (Fig 1).

The 45-year-old man (case 2) with nonunion of the left femur underwent surgical stabilization of the femur, performed by one of us (L.F.A.P.), in March 1969. Immediately before operation he received ANRC high-purity AHF containing 1,350 units of factor VIII (Fig 2). Internal fixation of the fracture with cobalt-chromium alloy compression plates and screws was carried out. Bleeding increased toward the end of the procedure. During the last portion of the operation, 3,250 additional units of factor VIII (ANRC preparation) were administered and then a maintenance dose was given at 12-hour intervals. The patient did well until one week postoperatively, when pain developed in his left thigh. The hemoglobin concentration in the blood dropped from 10.7 to 9.2 gm/100 ml despite administration of 4 units of whole blood, and a large hematoma developed in the left thigh. He was immediately given 1,200 units of factor VIII, the remainder of our supply of the ANRC concentrate, and thereafter lyophilized glycine-precipitated fraction (2,000 units of factor VIII) to raise the factor VIII level to 100% of normal. The subsequent program is shown in Fig 2.

The amount of factor VIII concentrate given was sufficient to raise the patient's factor VIII level to 45% of normal immediately before operation and to 100% shortly after. When the large hematoma developed beneath the operative site on the seventh postoperative day, the patient's factor VIII level was only 15% of normal. Although bleeding promptly stopped when the factor VIII level was raised, this hemorrhage did prolong his hospitalization.

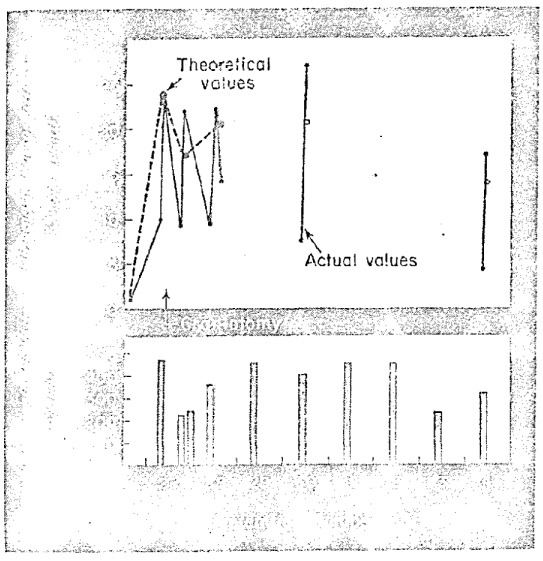
The 25-year-old man with granulomatous colitis and gastrointestinal bleeding had extensive ulceration of the terminal portion of the ileum and proximal two thirds of the colon on exploration. Subtotal colectomy (15 cm of ileum and 70 cm of colon) with ileorectosigmoidostomy was done, and microscopic studies revealed granulomatous colitis without submucosal hemorrhage. The patient was prepared preoperatively with lyophilized glycine-precipitated fraction containing 1,800 units of factor VIII and was subsequently given lyophilized glycine-precipitated fraction to maintain his factor VIII level at approximately 50% of normal for eight days postoperatively, when the factor VIII level was allowed to drop to as low as 25%.

Hemostasis was normal throughout the extensive procedure. For 5½ weeks postoperatively the patient had intermittent episodes of blood in the stool, but then he was able to return home.

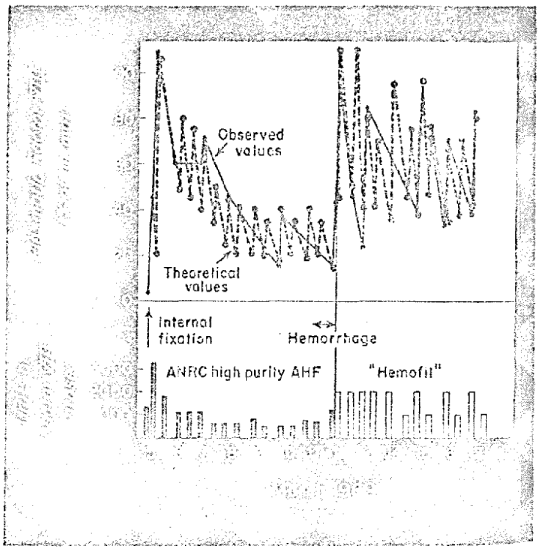
Patient 3 was readmitted to the hospital because of bleeding from the rectum and frequent passage of loose, bloody stools. Proctoscopic and x-ray film examination showed progression of the disease and ulcers at the site of anastomosis. Further resection of the bowel was necessary, and ileorectostomy was done after preoperative preparation with lyophilized glycine-precipitated fraction containing 2,000 units of factor VIII. Again hemostasis was excellent during the surgical procedure. The postoperative course was uneventful and no further bleeding occurred. The patient was dismissed from the hospital two weeks after operation, during which he had received maintenance doses of lyophilized glycine-precipitated fraction.

The 10-year-old boy (case 4) with equinovarus deformity of the right foot underwent a surgical procedure in May 1968 for lengthening of the Achilles and other tendons of the right foot, resection of the posterior tibial tendon, and multiple joint capsulotomies with insertion of multiple Steinmann pins. He was prepared preoperatively with 985 units of the ANRC preparation of factor VIII, which increased the plasmatic level of factor VIII to 75% of normal (Fig 3). He was given this preparation in a dose of 500 units every 12 to 24 hours for more than two weeks postoperatively. Periodic postoperative debridement and dressing changes with the patient under general anesthesia did not

1.--Data on factor VIII-deficient patient who received cryoprecipitate during and after craniotomy for subdural hematoma.



2.--Data on factor VIII-deficient patient who received ANRC high-purity antihemophilic factor and lyophilized glycine-precipitated fraction during and after operation for internal fixation of ununited femoral fracture.

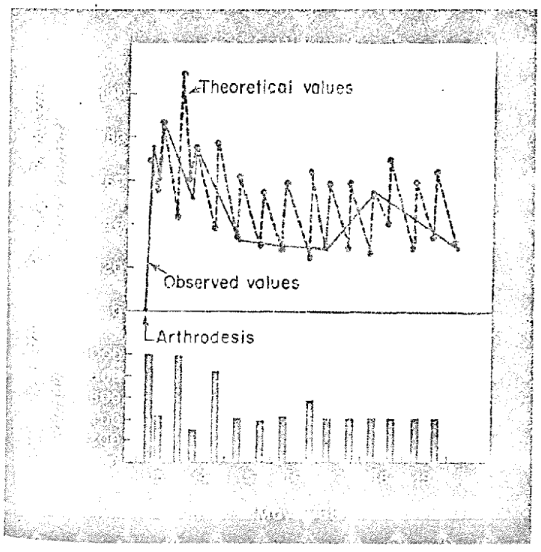


cause bleeding. Despite frequent debridement and meticulous care of the wound, enough necrosis of the overlying skin occurred (secondary to tension and ischemia) to require skin grafting.

Patient 4 underwent pedicle skin grafting six weeks after his initial operation. He was prepared with the ANRC factor VIII, so that a factor VIII level of 85% of normal was achieved. This level was maintained postoperatively with this preparation for one week, and with cryoprecipitate in doses of 100 to 250 units of factor VIII daily for an additional week. No bleeding occurred during or after the procedure. The patient could now get around much better because of the stable right ankle, and at last report could ride his bicycle.

The 22-year-old man (case 5) with painful hemophilic arthritis of the left knee underwent compression arthrodesis of the left knee and fixation with multiple compression plates and screws performed by L.F.A.P. in January 1968. He was given 1,000 units of factor VIII (cryoprecipitate) immediately before and 1,000 units during operation, and no unusual bleeding occurred. He was given a maintenance dose of cryoprecipitate containing 800 units of factor VIII every 12 hours. About two weeks postoperatively, drainage from the wound and pain in the area developed, and a sizeable hematoma was noted at the operative site. The dose of factor VIII was increased and the pain and drainage gradually ceased. Again, approximately four weeks postoperatively, as the dosage of factor VIII was being tapered off, a second but less significant hematoma developed. This was promptly controlled by increasing administration of the cryoprecipitate.

3.—Data on factor VIII-deficient patient who received ANRC high-purity antihemophilic factor during and after performance of triple arthrodesis of the ankle.



Examination of the left knee eight weeks after operation showed complete bony union and the patient was eventually able to walk without pain in the left leg for the first time in many years. Ambulation progressed with physical therapy and he returned to normal daily activity without the use of brace or wheelchair. Unfortunately, he died several months later from complications associated with an automobile accident.

Comment

The seven operations performed on these patients with classic hemophilia represent three types of major surgery: orthopedic, abdominal, and neurosurgical. Three of the patients underwent orthopedic types of surgical procedures, which are the most common operations in hemophiliacs. The usual indications are trauma and extensive joint damage due to hemorrhage into the joint. Amputation, osteotomy, internal fixation, and reconstructive operations have all been carried out successfully in the hemophiliac, many of these procedures antedating the use of factor VIII concentrates.[8]

There have also been reports of neurosurgical procedures[9] and of major abdominal surgery performed when the only available source of factor VIII was blood or plasma. Since only modest increases of plasmatic factor VIII are possible with infusions of plasma, it is remarkable that such surgical procedures were successful. For example, in Silverstein's series,[9] four of 11 hemophiliacs survived craniotomy for intracranial bleeding.

In our experience, one can comfortably raise the factor VIII level of a hemophiliac from 0% to 5% with infusions of plasma, and to about 10% with the hazard of some degree of circulatory overload. These levels are believed to be adequate for preventing serious bleeding in the everyday life of the hemophiliac but are inadequate for hemostasis in major surgery.[10-12]

The demand for higher concentrations of factor VIII when a patient is operated upon has been met in very recent years by concentrates of plasmatic factor VIII. Although only a few of these are commercially available in the United States, a review of their status indicates that still more effective preparations will probably be forthcoming.

Factor VIII Concentrates.—Two basic principles have been followed in the preparation of factor VIII concentrates: (1) techniques designed to precipitate fibrinogen from plasma depend for their success upon the coprecipitation of factor VIII; such preparations can reduce the volume of fluid to be injected

into the patient about fivefold or tenfold for each unit of factor VIII compared with its concentration in plasma, and (2) techniques designed selectively to precipitate factor VIII with a minimum of associated fibrinogen; such preparations can be concentrated more than 100-fold, relative to plasma, are easier to reconstitute from the lyophilized state, and cause only a modest increase of fibrinogen in the recipient.

Fibrinogen-Factor VIII Precipitated Preparations.—Cryoprecipitation from fresh plasma has already been described. The precipitate, containing much of the fibrinogen of the plasma and usually less than half of the factor VIII, can be frozen and stored for many months with negligible loss of factor VIII activity.[13] Because cryoprecipitate is technically simple to prepare, conserves the other components of blood, and is a by-product of the routine collection of donor blood, it has become easily available to the clinician and surgeon. It has been the principal preparation used in the treatment of the hemophiliac over the past three years.[14]

A lyophilized preparation of cryoprecipitate is available commercially (Courtland Laboratory). It is stable at room temperature for as long as six months. The main complications are serum hepatitis and minor urticarial and febrile transfusion reactions, problems shared with nonlyophilized cryoprecipitates but of greater magnitude because of the much larger size of the donor pool.

Cohn's fraction I is a concentrate of fibrinogen precipitated by the addition of ethanol to plasma in the cold. The coprecipitated factor VIII makes it potentially useful in the treatment of hemophilia. A commercial preparation (from Merck Sharp & Dohme) is available and has been used effectively in hemophiliacs requiring surgical procedures.[15] There may be a high incidence of serum hepatitis and transfusion reactions accompanying its use.

"Purified" Concentrates of Factor VIII.—Various techniques have been developed to reduce the amount of fibrinogen in the factor VIII products.

Wagner and colleagues[5,6] found that the addition of glycine or alanine to plasma led to precipitation of the fibrinogen and factor VIII. Fibrinogen was then partially removed with a derivative of fuller's earth. A lyophilized glycine-precipitated fraction (Hemofil) is available commercially (Hyland Laboratories). It is stable at 4 C. Reconstitution is effected by adding water and warming but is very slow because of residual fibrinogen. The commercial vials contain about 100 units to 1,000 units of factor VIII per vial and are priced according to their factor VIII activity. Isoantibodies to major blood groups are present in this preparation[16] which is made from pooled plasma.

Alan Johnson, MD of the ANRC, has developed a series of purified factor VIII concentrates, each more refined than its predecessor. The principle of the high-purity concentrate involves precipitation of the factor VIII with polyethylene glycol. (The preparation we have used has not quite reached the stage of mass production.) It is lyophilized, stable, and dissolves very rapidly in water because it contains little fibrinogen.

Factor VIII Concentrates Available Abroad. An ether-precipitated fraction was developed by R. A. Kekwick, DSc, in Great Britain and has been available there, but not in this country for ten years. Extremely high concentrations of factor VIII have been prepared from porcine and bovine plasmas[17] but the incidence of hypersensitivity reactions has discouraged use of the animal concentrates in the United States.

In Sweden, Blombäck and Nilsson purified factor VIII by glycine precipitation from Cohn's fraction I.[18] Although containing a good deal of fibrinogen, it was freed of fibrinolytic enzymes. Because of its successful use in the treatment of hemophilia,[18] the Blombäck concentrate (fraction I-O) is widely used in Western Europe.

In Argentina, Pavlovsky et al discovered that tannates selectively precipitate factor VIII from Cohn's fraction I.[19] The fibrinogen-poor product, FI-OTA, is prepared and used in Argentina.

Hemostatic Levels of Factor VIII.—All factor VIII concentrates are expensive to produce and require large volumes of plasma in their preparation. In order to conserve these valuable therapeutic agents, we have reviewed our experience to find the lowest levels of factor VIII at which normal hemostasis can be maintained. Patient 1 presented no evidence of bleeding when factor VIII levels were as low as 5% to 10% of normal. Patient 2, on the other hand, bled postoperatively at an estimated factor VIII level of 15%. None of our patients, however, showed evidence of hemorrhage when the factor VIII levels were above 25% and it would seem reasonable to maintain the factor VIII at this level or higher until complete healing of the wound has occurred.

Higher levels of factor VIII must be achieved before operation, as evidenced by our experience with increased bleeding toward the end of the operation with patient 2. At the beginning of the operation his factor VIII level was 45%. It was estimated that at the end of the procedure the level was 30% if the half-disappearance time was normal, and it should have been adequate for hemostasis. At the end of the procedure, however, the bleeding became more difficult to control, which suggested that the factor VIII level had fallen below 30%. One possibility is that factor VIII may be con-

sumed more rapidly during operation. It would seem best therefore to give enough factor VIII concentrate to raise the level to 75% or 100% immediately before operation and to repeat the dose at the end of the procedure.

The length of time for which replacement therapy needs to be maintained depends on the type of operation. In the ileocolostomy performed on patient 3, replacement therapy was continued for a total of 16 days, at which time complete healing had occurred. In orthopedic procedures, however, the period of therapy needs to be longer and our experience suggests at least three to four weeks. Both patients 2 and 5 bled postoperatively after orthopedic procedures when plasmatic factor VIII level was allowed to fall below hemostatic levels because supplies of the concentrates were running short. In both instances the resultant bleeding caused a prolongation of the hospital stay. It should be emphasized that after orthopedic operations pain at the operative site is the earliest indication of bleeding.

In our experience the administration of factor VIII concentrates every 12 hours produces adequate hemostasis and is more convenient than more frequent administration of smaller doses. The therapy was controlled by performing assays of factor VIII levels twice daily initially and then once every day or two.

The currently available factor VIII concentrates allow more effective treatment of the usual bleeding problems with classic hemophilia[20] and almost any type of indicated surgical procedure to be undertaken. It is to be emphasized that such operations should be undertaken only if indications are unequivocal and in a center where the necessary clinical, surgical, and laboratory facilities are available. Although the factor VIII concentrates have opened new surgical avenues, expert surgical technique, clinical experience with hemophilia, and a modern blood bank and coagulation laboratory are essential prerequisites before surgical treatment of any type is undertaken in the hemophiliac.

Alan Johnson, MD, and Tibor Greenwalt, MD, provided the American National Red Cross "high-purity" AHF.

Collin S. MacCarty, MD, performed the surgery on patient 1; O. H. Beahrs, MD, performed the exploratory surgery on patient 3; and H. A. Peterson, MD, performed the operation to lengthen the tendon and J. N. Simons, MD, performed the skin grafts on patient 4.

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MEDICAL INTELLIGENCE

CURRENT CONCEPTS

Treatment of Hemophilia with Factor VIII Concentrates*

PETER R. DALLMAN, M.D.,† AND JUDITH GRAHAM POOL, PH.D.‡

THE treatment of hemorrhagic episodes in hemophilic patients requires administration of the coagulation protein that is deficient — namely, antihemophilic globulin (factor VIII), derived from the plasma of normal blood donors. Recently, two concentrated preparations of factor VIII have become increasingly available,[1,2] and their clinical effectiveness has been confirmed.[3-6] Our purpose is to review the characteristics of these preparations and to describe the changes in our management of hemophilic patients resulting from the availability of one of these concentrates during the past two years.

The treatment of hemophilia until very recently was dependent almost entirely upon fresh-frozen and lyophilized plasma. Both these preparations retain all the protein present in the donor plasma and therefore have a high content of protein in proportion to factor VIII activity. As a result, the level of circulating factor VIII activity that can safely be attained is limited by the hazards of hypervolemia and heart failure. The first successful efforts to prepare concentrated materials were reported from abroad: bovine and porcine preparations from England,[7] and a human preparation from Sweden.[8] In this country there has not been much enthusiasm for use of factor VIII from animal sources since the development of resistance and a high risk of antigenicity have limited their application to single major bleeding episodes.[9] However, the Swedish process has been put into commercial production in this country by Merck Sharp and Dohme, and for several years this fibrinogen-rich fraction was the only source of a somewhat purified human preparation.[10]

The more potent factor VIII concentrates now in widest use are cryoprecipitated factor VIII[1] and glycine-precipitated factor VIII.[2] Although these concentrates have similar therapeutic advantages, they differ in many of their characteristics, which are compared in Table 1. In brief, the cryoprecipitate is prepared in blood banks from single units of blood, which can be reconstituted for subsequent use in other patients; usually, one blood donation is accepted by the blood bank in "payment" for four units of cryoprecipitate. The cryoprecipitate must be stored in a freezer, and no assay of potency can be provided with individual units. The glycine preparation is derived from large pools of plasma and is considerably more expensive at present. However, it has the advantages of stability under ordinary refrigeration, and a known potency for each dose. Neither preparation is suitable for treatment of coagulation deficiencies other than factor VIII; therefore, it is essential that the diagnosis be firmly established.

The clinical experience at Stanford has been entirely with cryoprecipitated factor VIII, but most of the conclusions should be equally applicable to the use of the glycine preparation. During the past two years almost all bleeding episodes in 21 children with hemophilia (factor VIII deficiency) were treated with cryoprecipitated factor VIII. Data partially derived from the 15 adults similarly treated under the direction of William P. Creger, M.D.,§ are included with his kind permission. All but 6 patients had severe hemophilia with less than 1 per cent of normal factor VIII activity. We have used a total of over 4000 units of type-specific cryoprecipitate without any discernible transfusion reactions; however, a mild febrile reaction has been reported in a recent paper.[3] Factor VIII assay was carried out by the method of Pool and Robinson,[12] with minor modifications.[13]

Units of cryoprecipitate were supplied by two regional blood banks and stored frozen at −20°C in flattened plastic bags. When cryoprecipitate was required, blood-bank personnel dissolved the number of units ordered by injecting about 10 ml of isotonic saline solution into each bag and then combining the solutions in a single pack, to assure uniformity of handling and to keep the risk of bacterial contamination at a minimum. The pack of concentrate was then administered to the patient by means of a standard filter-set to remove any particulate matter that might remain. The precipitates usually were readily dissolved, and solid material was rarely found on the filter. An alternate method of administration was to draw the concentrate from each of the original bags into a syringe and then to inject through a small filter unit (Fenwal) inserted between syringe and needle. For small (pediatric)

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TABLE 1. *Characteristics of Two Concentrates of Human Factor VIII.*

CONCENTRATE	OUTLINE OF PRODUCTION	HANDLING OF REMAINDER OF BLOOD OR PLASMA	POTENCY	POTENCY CONTROL
Cryoprecipitate	Individual units of freshly collected plasma frozen, cold thawed & centrifuged in closed plastic-bag sets in blood bank; separated cryoprecipitate, 2-3 ml in volume, frozen & stored at −20°C or below.†	Remainder of plasma poor in factor VIII may be returned to packed red cells to reconstitute 1 unit of whole blood or further fractionated to yield albumin, gamma globulin, etc.†	Factor VIII purified 16 ± 7 times on protein basis as compared to fresh normal plasma†	Individual u vary in po tency like units of fr frozen pla
Glycine preparation‡	Freshly collected plasma pooled from 20 donors added to glycine to yield concentration of 2.3 M; resulting precipitate dissolved in citrated saline solution, filtered & freeze dried in 30-ml units, which are stored under refrigeration at 4°C.[31]	Since usual source is plasmapheresis, red cells returned to donor; glycine must be removed from rest of plasma before further fractionation.	Factor VIII purified 20-30 times on protein basis as compared to fresh normal plasma[29]	Specific assa supplied fo each lot by manufactu

*1 unit = mean activity in 1 ml of normal fresh plasma.

†Or 1/4 blood donation.

‡Antihemophilic factor, Human (Hyland Laboratories).

volumes the syringe permitted more rapid infusion and minimized the problem of washing-out dead space in tubing, an important consideration when concentrated materials are being used.

TREATMENT SCHEDULES

The potency of the cryoprecipitate units has made certain alterations in treatment schedule possible, and has permitted a greater emphasis on outpatient care. With the use of fresh-frozen plasma, intervals as brief and four, six or eight hours between infusions have been necessary to attain reasonable therapeutic levels of factor VIII activity without the risk of hypervolemia. The low volume and protein content of cryoprecipitates permit any desired range of factor VIII activity to be attained with the initial dose. Maintenance of the appropriate factor VIII activity is then possible with intervals of 12 and even 24 hours between treatments. Figure 1 shows the characteristics of various therapeutic intervals for the maintenance of factor VIII activity in excess of 15 per cent for a one-day period. A 12-hour half-disappearance time for factor VIII is assumed in a hypothetical 30-kg patient. The value approximates the actual half-disappearance time of 13.1 ± 4.8 (SE of mean) hours previously determined in 41 monitored infusions.[1] The six-hour treatment interval results in very little saving in concentrate as compared to the 12-hour interval, and the postinfusion peak factor VIII activity is about 25 per cent below that achieved with a 12-hour interval. The six-hour and eight-hour intervals, therefore, appear to offer few advantages unless prompt hemostasis is

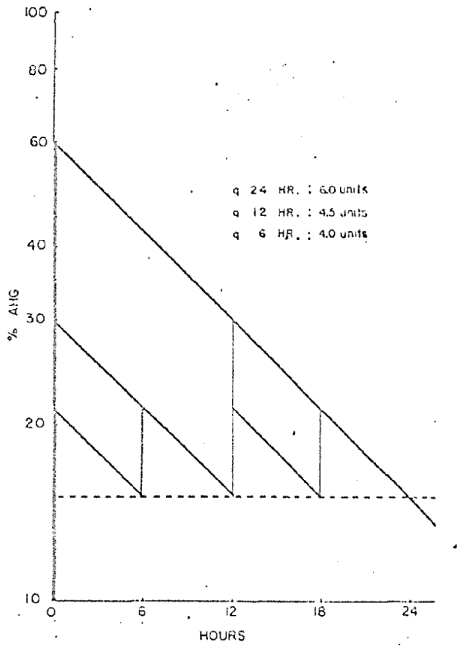

FIGURE 1. *Requirements of Factor VIII (AHG) for Maintenance Circulating Level in Excess of 15 Per Cent for a Twenty-Four Hour Period.*

The requirements and dose response for a single infusion are compared in twelve hour and six hour regimens. Units refer to the number of units of cryoprecipitate required in a hypothetical 30 patient, assuming an average dose response and a twelve-ho half-disappearance time.

TABLE 1 *(Concluded).*

CONCENTRATE	STORAGE STABILITY	PREPARATION FOR ADMINISTRATION	DATA ON INDIVIDUAL THERAPEUTIC UNIT				
			FACTOR VIII*	TOTAL PROTEIN	FIBRINOGEN	GLYCINE	COST
			units	*g*	*g*	*M*	*S*
precipitate	May be stored for at least 1 yr at −20°C without significant loss of potency	5-10 ml of isotonic saline solution added to each cryoprecipitate unit, & dissolved units combined into single pack or syringe & administered through filter‡	128 ± 58§	0.55§	0.19§	0.0§	ca 6.25†
cine preparation‖	May be stored for at least 1 yr at 4°C without significant loss of potency	30 ml of sterile water added to each vial; administration set with filter provided by manufacturer.	200-326¶#	0.66-0.7¶#	0.54-0.6¶#	0.1¶	40.00

unit = mean activity in 1 ml of normal fresh plasma.
† 1/4 blood donation.
‡ Antihemophilic factor, Human (Hyland Laboratories).

achieved or it is suspected that the initial dose inadequate.

ne characteristics of a 24-hour treatment interval particularly pertinent to the patients suitable for patient treatment. Because of the semilogarithmic acter of the decay, the 24-hour interval requires per cent more concentrate per day than the 12- r regimen to maintain the activity of factor VIII ve 15 per cent. The advantages of the 24- r regimen are a peak activity that is twice igh initially when the need for hemostasis is test and a substantial saving in staff and patient . There are, however, variations in disappear- t of the infused factor VIII. These varia- s become most evident with the 24-hour regi- during which a post-treatment activity of 60 cent, for example, may decay to 7.5 per cent at ours in one patient but only 30 per cent in another.

24-hour regimen, therefore, is inappropriate serious bleeding episodes when continuous factor VIII activities must be depended upon. ith inpatients we have most frequently em- ed the 12-hour regimen, using one unit of precipitate for 6 kg of body weight initially, owed by one unit per 12 kg every 12 hours, as a h guide to dosage. With this regimen, the aver- initial peak factor VIII activity is 51 ± 23 per (n = 70). The response obtained in children not differ significantly from that observed in ts, and factor VIII activity was usually main- d above 10 per cent, in the range that is ade- e for control of most hemorrhagic episodes. ng to the ease of administration of the concen- trate, more of our patients have recently been treated in the outpatient department for mild periarticular and soft-tissue hemorrhages that do not appear to be dangerous. They are generally seen again on the following day, but often no more than the first infusion is required. The rapidity and ease of outpatient treatments have appeared to induce patients to come in early in the course of hemorrhagic episodes. We encourage these early visits in the hope that prompt treatment will help reduce the degree of residual damage, particularly after joint hemorrhages. In the inpatient services the 12-hour and 24-hour regimens have reduced the demands upon the house staff and nursing personnel. Few infusions have had to be given at awkward hours; in addition, the frequent observation of infusion rate and vital signs previously required during plasma infusion is not necessary with the use of the cryoprecipitate.

VARIATION IN THE POTENCY OF THE CONCENTRATE

A major disadvantage of the cryoprecipitate, already alluded to, is the variation in potency from unit to unit. The factor VIII activities of normal donors range from 50 to 200 per cent of the mean level.[14] Simple freezing and thawing of plasma results in a 20 per cent loss of activity, thus reducing the levels in frozen plasma to a range of 40 to 160 per cent of the mean; since the cryoprecipitate yields only about 70 per cent of this activity, the final product will have a range of approximately 28 to 112 per cent of the mean factor VIII content of one unit of blood.[1] In the estimation of the postinfusion

level this variability is of less concern in large patients, who require enough units to make good averaging likely. In infants, however, because of the uncertainty regarding potency of a single unit, we frequently double the calculated dose.

The assay of postinfusion levels of factor VIII is not routine on our service. However, if cessation of bleeding is not prompt, or if any type of elective procedure is planned, some objective index of circulating factor VIII activity becomes very important. If a quantitative factor VIII assay is not available, either the activated coagulation time of Hattersley[6] or the partial thromboplastin time as modified by Proctor and Rapaport[15] may be used as a guide to therapy.

Elective Procedures

Surgery and dental procedures continue to pose difficult problems in spite of the use of concentrate. In both cases, one must still be prepared for the possible need to provide therapeutic factor VIII levels for 10 or more days after the procedure. Such levels must often be maintained above the 10 to 20 per cent that is adequate for the control of most spontaneous bleeding.[16] No elective surgical procedures were undertaken unless an ample supply of cryoprecipitate was available and before the responsiveness to treatment with factor VIII was ascertained with a trial infusion. Two of our patients had mild circulating factor VIII inhibitors. It was hoped that high doses of concentrate might overcome the effect of the inhibitor and provide brief periods of elevated factor VIII level in these patients. However, as much as one unit of cryoprecipitate per 2 kg of body weight did not result in a detectable increase in circulating factor VIII activity, and we therefore did not assume the risk of performing an elective procedure in either patient. Their hemorrhagic episodes, however, eventually resolved during therapy, and we continued to treat them with concentrate. Neither patient had an increase in inhibitor titer after therapy, and their low titers remained remarkably constant and independent of treatment for many months.

Discussion

If most units of routinely collected blood were processed, the potential supply of cryoprecipitate would permit programs of prophylactic therapy of hemophilia. However, such treatment would probably still use the intravascular route since experiments with intramuscular injection have shown recovery in the circulation of less than 10 per cent of the administered activity.[17]

Until now our experience with factor VIII cryoprecipitates has been very encouraging in facilitation of treatment and reduction of morbidity. The potential of regular prophylactic administration of factor VIII is still remote, but such a course is theoretically feasible.

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BRIEF RECORDING

Arterial Oxygenation during Ventilatio with the Ambu Self-Inflating Bag*

Morris L. Heller, M.D.,† T. Richard Watson, Jr., M.D.,‡ and Denis S. Imredy, Ph.D.§

A SIMPLE ventilator (the Ambu), first desc by Ruben,[1] has proved invaluable in resu

*From the Department of Anesthesiology, Hitchcock Clinic, Cardio-Pulmonary Laboratory, Hitchcock Foundation (address requests to Dr. Heller at the Hitchcock Clinic, Hanover, N.H.

Supported by a program project grant from the New Ham Tuberculosis and Health Association.

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‡Assistant clinical professor of surgery, Dartmouth Medical S director, Cardio-Pulmonary Laboratory, Hitchcock Foundation.

§Research associate, Hitchcock Foundation.

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SECTION 3. METHOD OF MANUFACTURE

3.1 Description of the Manufacturing Process.

Stage 1

~~(a)~~ Frozen plasma is removed from the collection bags ~~by~~ by thawing. ~~Hot water is used to remove from the bags~~ The frozen plasma is crushed and pooled and allowed to reach 3°C. Samples are taken for assay to insure freedom from hepatitis associated antigen.

Stage 2 (b) The cryoprecipitate is removed from the pool and ~~washed~~ dissolved in buffer solution containing glycine, sodium chloride and ~~sodium~~ sodium heparin.

Stage 3 (c) The buffer cryoprecipitate is ~~absorbed on~~ treated with sterilised non-reactive aluminium hydroxide and centrifuged. The centrifugate is buffered with sodium citrate and sodium heparin, filtered, sterile filtered, vialed and lyophilised.

Stage 4 (d) The final product is K- checked for hepatitis associated antigen.

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SECTION 2. DESCRIPTION.

A white to very pale yellow lyophilised cake in a vial with vacuum. ~~Filled with~~ The vial is fitted with a blue seal.

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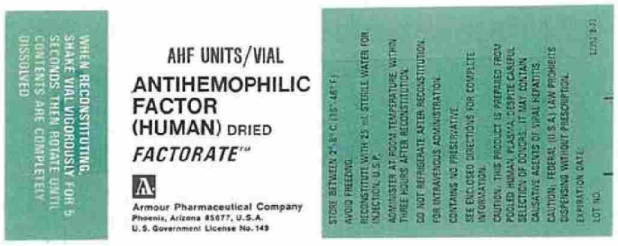
51



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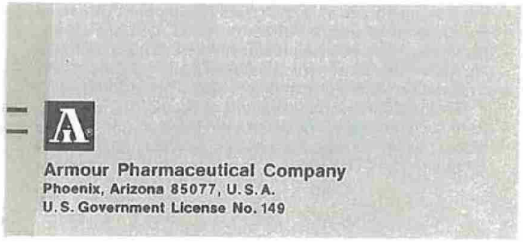

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ADDITIONAL INFORMATION

As a consquence of the application to the U.S. Dept. of Health to supply FACTORATE, a product licence was obtained for Armour Pharmaceutical Company and licence number 149 was issued on the 18th September 1972. This Licence No. 149 is subject to the following labelling requirements:-

1) Store between 2-8°C.
2) Avoid freezing
3) Reconstitute with 25 ml Sterile Water for Injection USP
4) Administer at room temperature within 3 hours after reconstitution
5) Do not refrigerate after reconstitution
6) Contains no preservatives
7) Caution: This product is prepared from pooled human plasma. Despite careful selection of donors, it may contain causative agents of viral hepatitis.
8) Caution: Federal law prohibits dispensing without prescription.

A copy of the vial labels outer carton and package insert are included on the previous pages 49 - 51.

No evidence of haemolysis or hepatitis has been recorded since the product was introduced.

REVISED PACKAGE INSERT LEAFLET

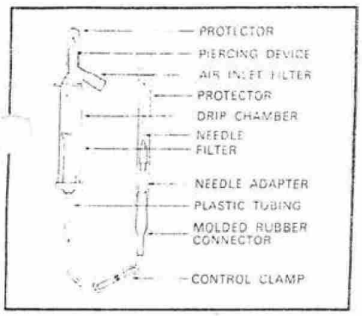
ANTIHEMOPHILIC FACTOR (HUMAN) DRIED *FACTORATE®*

Armour Pharmaceutical Company
Phoenix, Arizona 85077, U.S.A.
U.S. Government License No. 149

ARMOUR PRE-COILED I.V. ADMINISTRATION SET

NOTE:
This IV set has recently been modified. Read directions on back panel carefully before use.

TO OPEN PUSH IN AND TEAR DOWN



LOT NO.

ONE STERILE-DISPOSABLE SET
READY TO USE

INTRAVENOUS ADMINISTRATION SET

WITH
19 GAUGE—
1½ INCH NEEDLE

DIRECTIONS FOR USE:

1. Examine set to identify components and assure that end protectors are in place.
2. Remove outer seal from solution bottle and sterilize top of rubber stopper with antiseptic solution. Let dry.
3. Position clamp 6 to 8 inches from needle adapter end of set. Then close clamp.
4. Remove plastic protector from piercing device. With solution bottle in upright position, push the piercing device quickly through the outlet diaphragm in rubber stopper. (Push straight in, do not twist.)
5. With drip chamber partially compressed, invert bottle and release pressure on drip chamber. Suspend from suitable stand. Do not remove air inlet filter.
6. Open clamp and allow solution to displace air in tubing and needle. Squeeze flexible drip chamber to obtain desired solution level. Then close clamp.
   NOTE: Drip chamber should not be completely filled.
7. Remove plastic IV needle protector.
8. Proceed with venipuncture. Slowly open clamp and adjust for desired flow rate. (Approximately 10 drops = 1 ml.)
   NOTE: Add supplementary medication by injecting through raised portion of molded rubber connector. Do not inject into plastic tubing.

THE FLUID PATH, NEEDLE AND END CONNECTORS ARE STERILE, PYROGEN-FREE AND NON-TOXIC.
CAUTION: DO NOT USE IF DAMAGED OR IF END PROTECTORS ARE MISSING OR NOT IN PLACE.

DIAGRAM AND DIRECTIONS FOR USE ON BACK OF CARTON.

CAUTION: FEDERAL (U.S.A.) LAW RESTRICTS THIS DEVICE TO SALE BY OR ON THE ORDER OF A PHYSICIAN.

DO NOT OPEN UNTIL READY TO USE.

STERILE—DISPOSABLE

MANUFACTURED FOR
Armour Pharmaceutical Company
Phoenix, Arizona 85077, U.S.A.

ONE STERILE-DISPOSABLE SET
INTRAVENOUS ADMINISTRATION SET

A-134
12347/11-74

ARMOUR000187

Despatched to DHSS at a later date than original application
to replace that given on page 5).

REVISED PACKAGE INSERT LEAFLET

STORAGE

FACTORATE® and diluent are to be stored at refrigerator temperature (2°C.-8°C.). Freezing may damage the container for the diluent.

HOW SUPPLIED

Antihemophilic Factor (Human)—FACTORATE® is supplied in single dose vials (AHF activity is stated on label of each vial) with a vial of diluent and sterile needles for reconstitution and withdrawal.

REFERENCES

1. Treatment of Classical Hemophilia: The Use of Fibrinogen Rich in Factor VIII for Hemorrhage and for Surgery. McMillan, Diamond and Surgenor: New Eng J Med 265:224, 277, 1961.
2. Treatment of Hemophilia with Factor VIII Concentrates. Dallman and Pool: New Eng J Med 278:199, 1968.
3. Antihemophilic Factor VIII in Hemophilia. Mazza, J.J., et al.: JAMA 211:1818, 1970.

REVISED—SEPT., 1975 IBM 12358

ANTIHEMOPHILIC FACTOR (HUMAN) DRIED FACTORATE®

Armour Pharmaceutical Company
Phoenix, Arizona 85077, U.S.A.
U.S. Government License No. 149

1. OVERT BLEEDING—Initially 20 units per kg. of body weight followed by 10 units/kg. every eight hours for the first twenty-four hours and the same dose every twelve hours for 3 or 4 days.
2. MUSCLE HEMORRHAGES—
   a. Minor hemorrhages in extremities or non-vital areas: 10 units per kg. once a day for 2 or 3 days.
   b. Massive hemorrhages in non-vital areas: 10 units per kg. by infusion at 12 hour intervals for two days and then once a day for two more days.
   c. Hemorrhages near vital organs (neck, throat, subperitoneal), 20 units per kg. initially; then 10 units per kg. every 8 hours. After two days the dose may be reduced by one-half.
3. JOINT HEMORRHAGES—10 units per kg. every eight hours for a day; then twice daily for one or two days. If aspiration is carried out, 10 units per kg. just prior to aspiration with additional infusions of 10 units per kg. eight hours later and again on the following day.
4. SURGERY—Dosages of 30 to 40 units per kg. body weight prior to surgery are recommended. After surgery 20 units per kg. every eight hours should be administered. Close laboratory control to maintain the blood level of AHF above 40% of normal for at least ten days postoperatively is suggested. As a general rule one unit of AHF activity per kg. will increase by 2% the circulating AHF level. Adequacy of treatment must be judged by the clinical effects—thus the dosage may vary with individual cases.

WARNING

Antihemophilic Factor (Human)—FACTORATE® is prepared from human plasma, each donation of which has been found negative for Hepatitis B Surface Antigen (HBsAg) by the radioimmunoassay (RIA) method. In addition, this lot, after reconstitution as directed in this insert, has been tested and found negative by the RIA method. However, since no completely reliable laboratory test is yet available to detect all potentially infectious plasma donations, the risk of transmitting viral hepatitis is still present.

SIDE EFFECTS

Products of this type are known to cause mild chills, nausea or stinging at the infusion site.

PRECAUTIONS

FACTORATE® contains low levels of group A and B isohemagglutinins. When large volumes are given to patients of blood groups A, B, or AB, the possibility of intravascular hemolysis should be considered.

CONTRAINDICATIONS

There are no known contraindications to antihemophilic factor.

ARMOUR000188

ARMO0000002_0066

REVISED PACKAGE INSERT LEAFLET

ANTIHEMOPHILIC FACTOR (HUMAN) FACTORATE®

FOR INTRAVENOUS USE

Antihemophilic Factor (Human)—FACTORATE® is a stable lyophilized concentrate of Factor VIII (AHF, AHG) prepared from pooled human plasma intended for use in therapy of classical hemophilia (Hemophilia A).

A hereditary disorder of blood coagulation occurring almost exclusively in males, Hemophilia A results in profuse bleeding in joints, muscles or internal organs as a result of minor trauma. The disease appears to be due to a deficiency of a specific plasma protein, antihemophilic factor. FACTORATE® provides temporary replacement of the missing clotting factor.

Affected individuals frequently require therapy following minor accidents. Surgery, when required in such individuals must be preceded by temporary corrections of the clotting abnormality with fresh plasma transfusions, or by injections of Factor VIII concentrates. Obvious advantages of the use of concentrates of Factor VIII over plasma are the avoidance of hyperproteinemia and possible kidney dysfunction resulting from large volume transfusions.

Several different concentrations of Factor VIII have been used successfully. These range from Fraction I of Cohn to highly purified, potent preparations. Antihemophilic Factor (Human)—FACTORATE® is in an intermediate category,

being a purified cryoglobulin with much of the fibrinogen, as well as other plasma proteins, removed. When stored as directed, it will maintain its labeled potency for the dating period indicated on the label.

Upon reconstitution, Antihemophilic Factor (Human)—FACTORATE® contains 5 to 10 times as much Factor VIII as does an equal volume of plasma. Thus, it may be used to correct deficiencies in Factor VIII levels without overloading the circulatory system.

COMPOSITION

FACTORATE®: Each vial contains the labeled amount of antihemophilic activity in AHF units. (One AHF unit is the activity found in one ml. of fresh pooled human plasma). Each vial also contains sufficient sodium chloride to make the reconstituted solution approximately isotonic when Sterile Water for Injection, U.S.P., 25 ml., is added.

RECONSTITUTION

1. Warm both diluent and FACTORATE®, in unopened vials to room temperature [not above 37° C. (98° F.)].
2. Remove caps from both vials to expose central portions of the rubber stoppers.
3. Treat surface of rubber stoppers with antiseptic solution and allow to dry.
4. Using aseptic technic insert one end of the double-end needle into the rubber stopper of the diluent vial. Invert

the diluent vial and insert the other end of the double-end needle into the rubber stopper of the FACTORATE® vial. Diluent will be drawn into the FACTORATE® vial by vacuum. (In order to assure transfer of all the diluent adjust position of the tip of the needle in the diluent vial to inside edge of the diluent stopper).

5. Remove diluent vial, then the double-end needle, from FACTORATE® vial.
6. Gently rotate FACTORATE® vial until contents are dissolved. **DO NOT SHAKE VIAL.** Vigorous shaking will prolong the reconstitution time. Complete solution usually takes less than 5 minutes. The solution is now ready for administration.

ADMINISTRATION

INTRAVENOUS INJECTION

Plastic disposable syringes are recommended with FACTORATE® solution. The ground glass surfaces of all-glass syringes tend to stick with solutions of this type.

1. Using aseptic technic, attach the filter needle (blue cap) to a sterile disposable syringe (10 ml.-25 ml.). Insert filter needle into stopper of FACTORATE® vial; inject air and withdraw the reconstituted solution from the vial.
2. Discard the filter needle and attach suitable infusion set or intravenous needle (not supplied).

3. Administer solution intravenously at a rate comfortable for the patient. Usually 25 ml. may be given in about five minutes.

INTRAVENOUS INFUSION

1. Prepare solution of FACTORATE® as described under Reconstitution.
2. Slip the vial into the plastic suspension sleeve. Keep vial upright.
3. Follow the Directions for Use accompanying the Intravenous Administration Set.
4. If more than one vial is to be administered to the same patient, the administration set may be transferred to a second vial prepared according to steps 1 & 2 above.
5. When infusion of FACTORATE® is complete, the administration set may be flushed with sterile isotonic saline to avoid loss of any of the reconstituted solution.
6. After use, discard administration set, needles and vials together with any unused solution.

DOSAGE

Antihemophilic Factor (Human)—FACTORATE® is for intravenous administration only. Although dosage must be individualized according to the needs of the patient (weight, severity of hemorrhage, presence of inhibitors) the following general dosages are suggested: