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APPLICATION FOR A VARIATION
TO PL 0231/0038

FACTORATE

JANUARY 1985 **AP000402**

ARMOUR001397

APPLICATION FOR A VARIATION TO PL 0231/0038

FACTORATE

(Antihaemophilic Factor)

C O N T E N T S PAGE NO.

* Information already presented in full in PL Application Heat Treated High Potency Factorate (PL 0231/0072)

MEDICINES ACTS 1968 AND 1971

FORM MLA 221
(Revised 1984)

APPLICATION TO CHANGE TO PRODUCT LICENCE

Licence Number: PL 0231/0038 Product Name: FACTORATE

Name and Address of Licence Holder: ARMOUR PHARMACEUTICAL COMPANY LIMITED,
ST. LEONARD'S HOUSE,
ST. LEONARD'S ROAD,
EASTBOURNE,
EAST SUSSEX.

Telephone Number: (0323) 21422
Your reference: R84/286

Please indicate if you have changed or propose to change any of the following:

- ☐ Name of Product
- ☐ Pharmaceutical Form
- ☐ Manufacturer
- ☐ Date of Expiry of Licence
- ☐ Active Ingredients
- ☐ Indications
- ☐ Dosage
- ☐ Contraindications and warnings
- ☐ Method of Retail Sale and Supply
- ☐ Activities covered by Licence
- ☐ Assembler
- ☐ Arrangements for Storage
- ☐ Container
- ☐ Shelf Life or Storage Precautions
- ☑ Method of manufacture
- ☐ Quality Control Procedures
- ☐ Finished Product Specification
- ☐ Constituent Specification
- ☐ Excipients
- ☐ Supplier of Active Ingredients
- ☐ Other (Specify)

On the attached sheet, give the present particulars, the change or proposed change and the reason. Any supporting evidence should be attached to the application. Please indicate the number of volumes and number of copies sent. Where the licensee has already completed MLA 201R or the latest versions of MLA 201 or MLA 231 (ie Revised 1984) it would be helpful if 3 corrected copies of the appropriate page could also be attached.

For Licensing Authority Only:

Application dated.....
Received.....
Acknowledged.....
Stats ref.....
Code.....

Route:

Pharm:

Med:

Application Approved

Pharmacist:
Date:
Doctor:
Date:

AP000404

ARMOUR001399

Name of Product: FACTORATE Licence Number: PL 0231/0038

Address for reply:

Mr. C.J. Collins,
Armour Pharmaceutical Company Limited,
St. Leonard's House,
St. Leonard's Road,
Eastbourne, E. Sussex.

Give the present product particulars and proposed change. If the change refers to particulars on the Schedule of the product licence you should give them exactly as they currently appear on the licence and how you propose they should be stated (continue on a separate sheet if necessary). Please attach supporting evidence to the application and indicate the number of volumes and copies.

Present	Proposed
Method of Manufacture As currently detailed in the particulars submitted under PL 0231/0038.	The method of manufacture will be as currently undertaken but with an additional step involving heat treatment. The finished lyophilised vials from the current process will be subjected to a heat treatment in a water bath at an attained temperature of 60°C ± 1°C for a period of 30 hours.

I hereby make application for the above licence to be changed in accordance with the proposals given above and certify that the changes will not adversely affect the quality of the product.

Signed GRO-C Date 4th January 1989.

Status Regulatory Affairs Manager.

The licensing authority *consents to/acknowledges your request to change the product licence as outlined at 8 above.

Please retain this form with the formal documents relating to the product licence as evidence of *approval/notification of the change.

Signed: Date:

A person authorised to sign
on behalf of the Secretary of
State for Social Services

*Delete as appropriate

AP000405

REASON FOR CHANGE/BACKGROUND STATEMENT

For some years work has been progressing at a number of centres particularly in the U.S.A. on the development of Factor 8 products with reduced risk of the transmission of hepatitis. Work at Armour Pharmaceutical Company, Kankakee, Illinois resulted in the development of a heat treatment stage incorporated as the final part of the process in the current production of intermediate potency Factorate (PL 0231/0038) and High Potency Factorate (PL 0231/0044). Initial studies revealed that heat treatment, a commonly accepted concept in the destruction of heat-labile viruses, was insufficient alone to prevent the transmission of Hepatitis B, although evidence existed to suggest that non-A non-B viruses might be removed by this process.

The process was approved by the F.D.A. for the U.S. market and by the B.G.A. for the German market. A Product Licence application was submitted 16th February 1984 for the High Potency Heat Treated Factorate (Ref. No. allocated PL 0231/0072). Earlier, a C.T.X. application was submitted for the intermediate purity product Factorate Heat Treated (C.T.X. 0231/0070A) on 11th August, 1983 in order to undertake a definitive study at U.K. Haemophilia Centres over an extended period of time in previously untreated patients to determine if infectivity of the product had been eliminated. Subsequent events indicated strongly that while the products were clinically effective, the heat treatment process employed did not achieve the desired objective in relation to NANB hepatitis, and the Product Licence application was withdrawn and the C.T.X. surrendered in November 1984.

During 1984 concern among U.K. physicians continued to grow over the A.I.D.S. situation. Isolation of what is thought could be the causitive virus was made by groups of workers in the U.S.A. and in France in the second half of 1984. H.T.L.V.III (human T-cell lymphoma-leukaemia virus) is the name given to the virus by the U.S. workers while the French group named the virus L.A.V. (lymphadenopathy associated virus). The viruses are probably the same. Assays for the detection of the virus itself are currently being developed while only relatively recently have tests for the H.T.L.V.III antibodies become available. Using the new tests L.A.V./H.T.L.V.III antibodies have been found in a high proportion of haemophiliacs but how this fact relates to the risk of contracting A.I.D.S. has yet to be established. The presence of antibodies implies previous exposure to L.A.V./H.T.L.V.III and hence the potential problem of A.I.D.S. is perhaps wider than originally perceived. The presence of antibodies also usually indicates immunity to infection and this may account, at least in part, as to why the risk of contracting A.I.D.S. is relatively low (around 1 in 1000). However, with investigation at an early stage this figure may be low.

The heat treatment process developed for Factorate and High Potency Factorate products in relation to NANB hepatitis looked as though it may be sufficient to destroy the L.A.V./H.T.L.V.III virus. A programme of work to assess the situation in relation to a number of heat-treatment processes and products has been undertaken by the U.S. Centre for Diseases Control (C.D.C.) in conjunction with the F.D.A. Bureau of Biologies (B.O.B.). It would appear that in the in-vitro situation 60^{o}C for 4 minutes will inactivate the virus in certain conditions and the information available on the work done to date is presented in this application.

All the indications are that the L.A.V./H.T.L.V.III virus is relatively heat-labile and that the heat treatment proposed for Factorate and Heat Treated Factorate ($60^{o}C$ x 30 hours) is effective in destruction of the virus. The products incorporating the heat treatment process have been widely used in the U.S.A., and in clinical study in the U.K. and the products have been shown to be clinically effective and appear to have a "safety in use profile" allied to the existing U.K. products. There have been an unprecedented number of 'named patient' requests and the situation with regard to such supply on the scale sought is totally unsatisfactory. It is clear that Haemophilia Centre Directors believe that a heat treated product, even though not proven over time to eliminate the A.I.D.S. risk virus is preferable to a non-heat treated product.

Studies at C.D.C. and our own in-house studies on the effectiveness of the heat treatment process for Factorate and High Potency Factorate are continuing. However, we believe the products with a heat treatment process as proposed are safe and effective. Furthermore we believe the heat treatment process proposed will render the existing products obsolete and thus apply for a variation to the existing Licences.

The data presented in this file should be considered in conjunction with the data presented in a similar variation for heat treated High Potency Factorate (PL 0231/0044) contained in a separate file, in view of the close similarity of the two products.

It should be stressed that much of the supporting data is the same.

APPLICATION FOR A VARIATION TO PL 0231/0038 - FACTORATE

1. CHANGE PROPOSED

Method of Manufacture

The manufacturing procedure is the same as that currently used for the product and as already discussed under the Licenced Particulars for PL 0231/0038. The following additional step is proposed.

After lyophilisation and sealing of the vials, the vials are brought to a temperature of 60°C ± 1°C in a water bath and held at this temperature for a period of 30 hours.

The remainder of the processing and assembly is unchanged.

2. QUALITY CONTROL OF THE FINISHED PRODUCT

The existing finished product specification (i.e. for each unit dosage presentation) will remain applicable and no changes are proposed. In-process specifications as currently applied will continue unchanged.

3. BATCH ANALYSES

Supporting evidence that the heat treatment process used does not affect Factorate material is provided by the results of batch analyses of three batches of Factorate before and after heat treatment, a copy of which is presented overleaf.

Potency assays according to normal methodology have been carried out on material from seven batches of Factorate (High Potency Product) before and after heat treatment. Six of the batches were heated (as proposed) at 60°C for 30 hours and one batch (TM 223) at 60°C for 72 hours. The results obtained were as follows:

Batch No.	Potency before Heating	Potency after Heating	% of Unheated
W 77405	1170	1150	98%
W 78105	1005	1020	101%
W 103306	1150	1095	95%
A 1607-020 (V 28602)	728	674	93%
A 1607-022 (U 26712)	782	689	88%
A 1607-024 (V 44106)	826	783	95%
TM 223	891	884	99%

Although the percentage estimate of potency (heated versus unheated) ranged from 88 - 101%, no statistical significance can be attached to these observed differences in view of the estimated confidence limits of the assay (95% c.l. = ± 80u/vial). All potency values were within the established limits of 80 - 125% for the assay. It was concluded that there was no significant loss of potency ascribable to heat-treatment.

COMPARISON OF ANALYSIS OF FACTORATE PRE AND POST HEAT TREATMENT

Test	X23102 Heat Treated		X24302 Heat Treated		X25203 Heat Treated	
Assay	200	195 (190)	200	225 (220)	200	210 (205)
Freedom from Abnormal Toxicity	Pass	Pass	Pass	Pass	Pass	Pass
Pyrogens	Pass	Pass	Pass	Pass	Pass	Pass
Heparin iu/vial	3	4	2	5	2	6
Total Protein mg/l	9.7	14.5	12.3	16.4	19.5	22.4
Clottable Protein mg/l	6.6	6.4	6.9	7.3	9.8	11.4
pH upon reconstitution	7.5	7.4	7.5	7.3	7.6	7.3
Solution time min	3	2	3	3	3	6
Issoagglutinins Anti-A	1:32	1:16	1:1	No measurable Titre	1:128	1:128
Anti-B	No measurable Titre	No measurable Titre	1:8	1:8	1:32	1:16
Hepatitis B_s Antigen	Negative	Negative	Negative	Negative	Negative	Negative
Citrate mM/litre	6	7	12	8	16	10
Moisture %	0.1	0.3	0.0	0.2	0.1	0.2

AP000410

ARMOUR001405

ARMO0000164_0009

4. STABILITY

A stability study has been conducted (and is on-going) with heat treated Factorate. The material has been stored at refrigerated termperature and 6 month results are presented. The report on the work is presented overleaf. Additionally data is provided on page 12 on the stability of a batch of Factorate to which has been added Hepatitis B immune globulin (equivalent to approximately 3 mg material per vial) which has then been heat treated in the proposed way. This material has been placed on accelerated stability study.

Further stability data on Armour anti-haemophiliac Factor are provided on the High Potency material in the accompanying file for a similar variation to PL 0231/0044.

Acute Stability of reconstituted Factorate before and after heat treatment

Three vials of High Potency Factorate (Batch TM 223) were heated at 60°C for 72 hours. After heating, two of the vials together with a vial of unheated control product were reconstituted with 30 ml Water for Injections and assayed over a period of 25 days. In addition a portion of each vial was diluted to a concentration of 1 unit/ml aseptically, using sterile citrate-saline buffer and assayed at the same times as the undiluted product.

The study was terminated at 25 days because A.H.F. activity in all three diluted samples had fallen to levels almost undetectable in the assay. Comparison of assays on heated and control unheated material showed no significant difference in potency of either concentrate or diluted material at any of the reference points. It was concluded that the heating procedure had not affected the stability of the product.

A copy of the 'in-house' report is presented on page 13.

SEC #83-19

RESEARCH AND DEVELOPMENT DIVISION

STABILITY OF HEAT-TREATED FACTORATE R,
GENERATION I, ARMOUR LOT W12011

AUGUST 1983

GRO-C

D.M. Lamontanaro, Ph.D.
SEC Chairman

APPROVED GRO-C

John E. Larembo, Ph.D.
Director

GRO-C

J. Newman, Ph.D.
Plasma Fractions Division

AP000412

SUMMARY

The data in this study show that Heat-Treated Factorate R, Generation I, is stable after 6 months storage at refrigerated conditions.

INTRODUCTION

This stability study was carried out to determine if heating Factorate R, Generation I, for a period of 30 hours at 60°C, a temperature which may be sufficient to destroy viruses, would affect the stability of Factor VIII:C biological activity.

EXPERIMENTAL

Factorate R, Generation I, Lot W12011, manufactured by Armour Pharmaceutical Company, Kankakee, IL and bottled in 50 ml vials was used for this study. A portion of the lot was heated for 30 hours at 60°C and the remainder was left unheated. Initially, the heated and unheated lyophilized stability samples were reconstituted by procedure, APC-1079 and assayed by procedure GOP 36/1981. Lyophilized samples were then stored at refrigeration. After six months they were observed for appearance characteristics and assayed against the in-house standard Anti-hemophilic Factor (AHF), Armour Lot K574121.

RESULTS AND DISCUSSION

The biological assay results and observed reconstitution times for the unheated and heated stability samples are listed on Table I. The appearances of the heated and unheated lyophilized stability samples were also visually recorded.

Initial assay data indicate that heating this material as discussed above did not cause noticeable changes in biological assay, reconstitution rates or appearance characteristics.

After 6 month storage at refrigeration both the unheated and heated Factorate R, Generation I stability samples did not show significant changes in biological activity, reconstitution rates or appearance characteristics.

These results support the conclusion that heat treatment of Factorate R, Generation I, does not have any initial effect on this material, nor does it affect the stability of the material stored six months at refrigeration, the labeled storage condition for this product.

TABLE I (1)

STABILITY OF HEAT-TREATED FACTORATE R, GENERATION I
LOT W12011, 200 UNITS/VIAL

SAMPLE	FACTOR VIII:C ASSAY U/vial PROCEDURE: GOP 36/1981 SPEC:		RECONSTITUTION TIME (3) PROCEDURE: APC-1079 SPEC: NLT 30 min.		APPEARANCE OF CAKE SPEC: White to pale yellow cake.	
	INITIAL	6 MONTHS (2)	INITIAL	6 MONTHS (2)	INITIAL	6 MONTHS (2)
UNHEATED	201	192	LT 5	LT 4	Conforms	Conforms
HEATED	197	184	LT 4	LT 4	Conforms	Conforms

(1) Data compiled by Plasma Fractions Division, Protein Biochemistry Department, Revlon Health Care Group, Tuckahoe, NY.

(2) Stored at refrigeration

(3) Reconstitution with Sterile Water for Injection.

AP000414

ARMOUR PHARMACEUTICAL COMPANY
KANKAKEE, ILLINOIS

12

STABILITY REPORT

Product: Antihemophilic Factor (Human), Heat-Treated, with Anti-HBs Added

Lot No.: X22602

Temperature: 2°-8°C, 15°-30°C, 37°C

Interval: 3 Months

TEST (1)	TEMPERATURE			
	Initial	2°-8°C	15°-30°C	37°C
Potency (AHF units/vial)	285	250	310	305
3 Hour Post-Reconstitution Potency (AHF units/vial)	305	295	--	310
Appearance	Pass	Pass	Pass	Pass
Solution Time (minutes)	2	5	3	3
Anti-HBs Titer (I.U./ml)	0.48	0.73	0.72	0.70

(1) TEST	ACCEPTANCE CRITERION	METHOD
Potency and 3 Hours Post-Recon. Potency	Not less than 150 units/vial	365
Appearance	Conforms to description	1396
Solution Time	Not more than 30 minutes	1079
Anti-HBs Titer	Not less than 0.25 I.U./ml	1442

AP000415

ARMOUR001410

ARMO0000164_0014

CONFIDENTIAL AND PROPRIETARY
NOT TO BE DUPLICATED

PROJECT: Factor VIII, Human, Hepatitis Safe

STUDY NO.: PFR-81-010

TITLE

Stability Study of Generation II Factorate which has been heated to 60°C for 72 hours.

OBJECTIVE: Comparison of the stability of heated Factor VIII to an unheated control. The comparison is made on the concentrate form as well as the concentrate diluted to 1 unit AHF/ml. The entire stability study is at room temperature.

SUBMITTED BY: Joseph Accumanno 9/17/81

REVIEWED BY: GRO-C 9/21/81

__/__/__

PLASMA FRACTIONS DIVISION
PROTEIN BIOCHEMISTRY DEPARTMENT

AP000416

Background:

Previous experiments have indicated that heating Generation II Factorate to 60°C for 24 hours may not be sufficient to destroy Hepatitis B. Therefore, a longer period of heating may be necessary. This experiment is designed to detect changes, if any, in AHF activity when Factorate is heated to 60°C for 72 hours in the dry state.

Outline of Experimental Approach

Three vials of Generation II Factorate were heated in a circulating water bath. After heating, one of the heated vials was stored in a -80°C freezer. Two heated vials and an unheated control were reconstituted with 30 ml of sterile water and assayed. Immediately upon reconstitution, each concentrate was diluted to 1 unit/ml using sterile citrate saline buffer and sterile vials, stored at room temperature and assayed over a period of 25 days.

Results:

Concentrate Vials
Total Units AHF/Vial

day #	unheated control	heated vial#1	heated vial#2	ave. of heated vials
0	891	861	906	884
5 hrs.	858	858	870	864
1	850	810	846	828
4	867	879	861	870
12	903	876	852	864
19	777	699	725	712
25	705	681	717	699

Vials Diluted to 1 Unit/AHF/ml
Total Units AHF/Vial

day #	unheated control	heated vial#1	heated vial#2	ave. of heated vials
0	876	891	882	887
5 hrs.	879	849	858	854
1	828	798	804	801
4	615	630	603	617
7	426	450	441	446
12	276	285	281	283
15	153	138	156	147
19	100	91	110	101
25	50	50	45	48

AP000417

ARMOUR001412

ARMO0000164_0016

Discussion:

The stability study was concluded because the AHF activity in all three diluted vials had fallen to a level which was almost undetectable in the assay system.

Conclusion:

The assay results have shown that heating Generation II Factorate to 60°C for 72 hours does not have an effect upon the AHF activity when compared with unheated Factor VIII stored in the same way.

Technical Details:

The vials of Factorate, Lot #TM223* were heated in a Precision circulating water bath. The temperature of the Factorate was monitored by the use of a probe in a dummy vial. At the end of the 72 hours, the vials were removed from the bath and placed on ice to rapidly cool them. An unheated vial of Factorate, Lot #TM223* was removed from a -80°C freezer and allowed to reach room temperature. Two heated and one unheated vial were reconstituted with 30 ml of sterile water.

Results of Reconstitution

	unheated control	heated vial#1	heated vial#2
Cake Color	white	white	white
Reconstitution Time	10min, 15sec	14min	13min, 30sec
Appearance	clear, colorless	clear, colorless	clear, colorless

After the vials were reconstituted, 1:40 dilutions of each concentrate were made using sterile vials, sterile buffer and sterile technique. The diluting buffer used was 4.2 gm sodium citrate, 7.5 gm sodium chloride, q.s. to 1 liter with water and pH 6.7. The assay system used for the experiment was the one stage PTT assay using Factor VIII deficient plasma as substrate.

* Final product lot number U22310

5. EXPERIMENTAL AND BIOLOGICAL STUDIES ON HEAT TREATED FACTORATE

5.1. The effect of heat treatment on thrombin activation

Thrombin has been shown previously to produce a pronounced increase in Anti-Hameophilic Factor activity, measured in a one-stage assay. This phenomenon may be of importance as an amplification mechanism in the intrinsic clotting cascade and consequently experiments have been carried out to determine whether heat-treated AHF retains this characteristic. Heat-treated material used in this experiment was prepared by heating at 70°C for 25 hours. Studies on the time-course of thrombin activation showed a peak of activation after 2 - 5 minutes incubation at which time the AHF activity increased twelve-fold over starting values. The effect gradually diminished with further incubation until at 15 - 20 minutes values were at or below initial results. Comparative tests with heated and non-heated material, using an incubation period of 2 minutes and measuring activity by a one-stage partial thromboplastin time test, showed no significant difference between the two materials.

The company report on this study is presented in the Appendix to this file, page 32.

5.2. Immunological testing in the rabbit

Immunological testing was carried out in order to determine whether heating of the complex protein of anti-haemophilic factor might lead to structural modification.

Antibodies to heated and non-heated Factorate were developed in New Zealand white rabbits (3 per group) by monthly subcutaneous injections of protein emulsions. The antibodies produced were then used in Ouchterlony gel diffusion and two-dimensional immuno-electrophoresis experiments in an attempt to detect the presence of new antigens. Results of both series indicated no difference in the patterns obtained for heated and non-heated material attributable to the heat-treatment. Similar tests with antisera specific for various plasma proteins, e.g. immunoglobulin, fibrinogen, albumin and transferren also failed to show any differences between the two products.

A copy of the full report is presented in the Appendix to this file, page 37.

A further study on the effect of heating on the proteins present in Factorate is presented in the Appendix to this file, page 43.

5.3. Half-life and recovery of untreated and heat treated Factorate in dogs

Studies on the half-life, recovery and various haematological parameters of heated and non-heated AHF were carried out in male dogs (weight 27.3 - 36.4 kg) with haemophilia A. One dog received unheated and two dogs heated material, infused at 8 ml/min over a period of 3.5 to 4 minutes. The total dose

administered was 770 units/dog. Blood samples were obtained by venepuncture at 15, 60, 90 minutes and 3, 5, 8 and 24 hours after infusion. Blood was collected into 3.8% citrate and divided, one part being used to prepare platelet-free plasma for measurement of F VIII C and F VIII RA levels. Blood samples were tested, in addition, for White Blood Cell Count, Haematocrit, Total Protein and Platelet Count.

Respiration rate, pulse rate and rectal temperature of the dogs were recorded at each test point.

Measurements of F VIII C indicated a rise in the level of this activity in all dogs at 15 minutes after dosing, the increases being 76% in the dog treated with unheated material and 258 and 134% in the two dogs receiving heated material. The F VIII activity of the dog treated with unheated material fell to a level below the initial value at 24 hours, whereas the levels in dogs treated with heated material were 43% and 34% above initial values at this time. Calculations of half-life from this data gave values of 7.8 hours for unheated material and 18.2 and 22.5 hours respectively for dogs receiving heated material. Recovery of exogenous AHF was 65% in the control dog (unheated material) and 97% and 81% respectively in the two dogs receiving heated AHF.

Plasma VIII RA activity increased in all animals at 15 minutes by 26% (control) and 46 and 51% (heated AHF) respectively. At 24 hours values in the control dog were 26% below baseline whereas those in dogs receiving heated AHF were elevated by 24% and at baseline value respectively.

Both AHF preparations produced decreases in haematocrit values but all values remained within the normal range for dogs of the age used (i.e. 35 - 53%).

The levels of blood proteins fluctuated in all dogs during the study with indications that AHF treatment might reduce these levels; however values were within normal range for proteins in dogs, i.e. 4.9 - 9.6 gm %, at all times.

AHF treatment produced fluctuations in levels of white cell count and platelets during the experiment. There were indications that heated material may have induced leukopenia although it was felt by the investigator that these reductions were not significant as fluctuations in white cells are not uncommon in haemophilic dogs. The fluctuations in platelet-count during the experiment were attributed to the stress of the experiment rather to the specific treatment administered.

Assessment of physiological parameters showed increase in respiration rate, apparently unrelated to treatment and possibly due to stress in all animals. Pulse pressure was reduced dramatically in one dog receiving heated material but only slightly in the other. The results for this latter dog and those of the control animal were all within the normal range of 105 - 150 mm Hg for dogs.

There were no dramatic changes in rectal temperature during the experiment. The temperature of the control dog fell slightly at 5 - 8 hours post dose but had returned to initial value by 24 hours. Rectal temperatures increased in both dogs receiving heated material during the period up to 3 hours post dose, by 1 - 1.2^{o}C, followed by a fall in temperature at 8 hours.

It was concluded that heat-treatment of AHF does not adversely affect the recovery or half-life of F VIII C nor F VIII RA in haemophilic dogs compared with standard material. Neither material altered haematocrit or total blood protein and although there were marked fluctuations in WBC and platelet counts following treatment, these responses did not appear to be drug related. Respiration rate, pulse rate and rectal temperature appeared to be little affected by either treatment.

The full report on this study is presented in the Appendix to this file, page 47.

5.4. Effect of heat-treatment of Factorate on heart rate and arterial blood pressure in anaesthetised dogs.

Eight male Beagle dogs weighing 9 - 11.3 kg, fasted overnight, were used in the study. Anaesthesia was induced with sodium pentathol (15 mg/kg) and maintained with α-chloralose (60 mg/kg). Four dogs were treated with control, unheated Factorate (High Potency) 100 u/kg and four with the same dose of material which had been heat-treated at 60°C for 24 hours, heart rate and arterial blood pressure were monitored throughout the study. All dogs received Water for Injections at an injection rate of 4.4 ml/min for 4.5 minutes prior to testing with AHF.

Infusion of Water for Injections had little effect on heart rate or arterial pressure, the maximum changes observed being a decrease of 15 beats per minute in one dog and an increase of 20 beats per minute in another for heart rate and an increase of 13 mm Hg blood pressure in one dog.

Administration of unheated AHF caused maximal increases of 14, 30 and 40 bpm in three dogs and a decrease of 5 bpm in the fourth. Arterial pressure changed slightly, the range of effect being a reduction of 7 mm Hg to an increase of 18 mm Hg. Heated AHF caused reduction of heart rate in two dogs by 16 and 20 bpm respectively and an increase of 6 and 20 bpm in the other two dogs. Mean arterial pressure was elevated in all dogs, increases ranging from 4 - 22 mm Hg.

It was concluded that infusion of AHF at 100 u/kg to anaesthetised dogs produced minor changes in heart rate and mean arterial pressure. These effects were usually immediate and transient in nature. Heat treatment of the material did not increase the incidence of these effects.

The full report of this study is presented in the Appendix to this file, page 79.

6. LAV/HTLV III VIRAL INACTIVATION STUDIES

As cited in the 'Background' statement in this application a programme of work has been undertaken by the Centre for Diseases Control in the U.S.A. on behalf of the Bureau of Biologics (F.D.A. agency). The C.D.C. has conducted studies on a number of heat treatment procedures in both a dry (lyophilised) state and a wet (solution) state with anti-haemophilic products. The full results are not available at present but will be made available to co-operating companies at the earliest opportunity, now scheduled for mid- to late January 1985. Armour Pharmaceutical Company has its own programme on-going within a similar time frame for confirmatory purposes.

However, some C.D.C. results have been made known to us in a letter dated 29th November 1984 from Dr. B.L. Evatt, Director, Division of Host Factors, C.D.C., Atlanta. A copy of the letter and relevant results are presented on the pages following. From the information presented the LAV/HTLV III virus is inactivated very quickly at a 60°C temperature; indeed even the lyophilisation process appeared to cause a certain amount of viral inactivation. A heating process under certain conditions involving a temperature of 60°C for 5 minutes appears to inactivate the virus. It is believed that the heat treatment used by manufacturers for hepatitis virus inactivation will adequately inactivate LAV/HTLV III virus. Additional work by the C.D.C. group is currently on-going and we will provide these data to the D.H.S.S. as soon as practicable.

However, we believe that on the basis of the studies so far undertaken the heat treatment process proposed for our product will inactivate the LAV/HTLV III virus.

Other Viral Inactivation Studies

A study has been carried out on Armour Pharmaceutical Company's, U.S.A., Factor IX product Prothar to investigate the viral inactivation effectiveness of dry heat treatment at 60°C for 30 hours on several enveloped viruses considered to be models for human blood borne infectious agents. They were Sindbis, Vesicula stomatitis and Pseudorabies viruses. The heat treatment process applied to 'spiked' product resulted in significant viral inactivation. The full report is presented on pages 23-29.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
Atlanta GA 30333

November 29, 1984

Fred Feldman, M.D.
uman Plasma Development
rmour Pharmaceuticals
Rt. 50, North
radley, IL 60915

Dr. Feldman:

hank you for your inquiry concerning experiments on heat inactivation of LAV in clotting concentrates performed at CDC. Several preliminary experiments ere undertaken to determine the approximate time needed for inactivation. he method for quantitation of viral inactivation is based on a double antibody recapture assay developed by Steve McDougal. This method will soon e published in the Journal of Immunologic Methods. I have enclosed a reprint of that assay for your information.

AV virus was added to the reconstituted factor VIII concentrate to achieve a inal concentration of 10^6 to 10^7 viable virus particles per ml. Glycine and sucrose were added for stabilization of protein and the material heated at various periods ranging from 1 minute to several hours at 60°C. Virus was apidly inactivated and no detectable virus grew in inoculated cultures after -4 minutes of heating. The detection of limit of viable viruses in these ssays is about 10^1/ml.

ubsequently two experiments were performed using lyophilized material. LAV virus was then added to reconstituted factor VIII material to achieve a final oncentration of about 10^6 per ml. That material was lyophilized in half ml liquots for 24 hours and subjected to assay for viable virus. At the end of lyophilization the unheated material contained a little more than 10^3 virus er ml. When heated for various periods of time at 60° no viable virus was etected after 4-5 minutes of heating. No data was available on residual moisture in these preparations.

he next experiment was performed using lyophilized material. Bulk plasma was obtained and LAV virus was introduced to achieve a final concentration of $10^{6.2}$ per ml. After lyophilization, viable virus in the reconstituted aterial was $10^{4.27}$ per ml. Material was heated at 68°C for periods of 24, 48, 60, 72, 96 hours. In all samples following heat treatment, no viable viruses were detected in assay system.

A second series of experiments were performed on factor VIII and factor IX concentrates obtained from another company. Virus was introduced into liquid concentrates and then lyophilized. Initial titer of virus in Factor VIII was $10^{5.26}$ viable viruses per ml and in Factor IX $10^{5.25}$. After lyophilization, the viral titer was $10^{3.68}$ in Factor VIII, and in Factor IX, $10^{4.4}$. After heating both groups at 60° for 24 hours, no viable viruses were detected in either factor VIII or factor IX concentrates.

Because LAV appeared to be extremently heat labile, we believe that the procedures presently used by manufacturers for heat treatment of hepatitis virus would adequately inactivate LAV virus. We are collaborating with the FDA on a generic study to examine the effects of heating concentrate preparations in liquid and dry form for various periods and heat ranges. The data from these studies will be made available as soon as possible.

I hope this information will be useful to you. If I can be of any further help, please do not hesitate to ask.

Sincerely yours,

GRO-C

Bruce L. Evatt, M.D.
Director
Division of Host Factors
Center for Infectious Diseases

cc: J. Steven McDougal

Enclosure

LAV INACTIVATION EXPERIMENTS (CONDUCTED BY CDC, ATLANTA)

		PRODUCT	CONDITION	HEAT TREATMENT	VIRUS TITER
1.	A.	RECONSTITUTED AHF-SOLUTION	GLYCINE, SUCROSE STABILIZERS	NONE	10^6 - 10^7/ML
1.	B.	RECONSTITUTED AHF-SOLUTION	GLYCINE, SUCROSE STABILIZERS	60° C/ 4 MINUTES	UNDETECTABLE*
2.	A.	RECONSTITUTED, LAV SPIKED AHF (10^6/ML)	REDRIED	NONE	10^3/ML
2.	B.	RECONSTITUTED, LAV SPIKED AHF (10^6/ML)	REDRIED	60° C/ 5 MINUTES	UNDETECTABLE*
3.	A.	BULK PLASMA SPIKED WITH LAV ($10^{6.2}$/ML)	FREEZE DRIED	NONE	$10^{4.27}$/ML
	B.	BULK PLASMA SPIKED WITH LAV ($10^{6.2}$/ML)	FREEZE DRIED	68° C/ 24 HRS.	UNDETECTABLE*
	C.	BULK PLASMA SPIKED WITH LAV ($10^{6.2}$/ML)	FREEZE DRIED	68° C/ 48,60,72,96 HRS.	UNDETECTABLE*
4.	A.	RECONSTITUTED, LAV SPIKED AHF ($10^{5.6}$/ML)	REDRIED	NONE	$10^{3.68}$/ML
	B.	RECONSTITUTED LAV SPIKED AHF ($10^{5.6}$/ML)	REDRIED	60° C/ 24 HRS.	UNDETECTABLE*
5.	A.	RECONSTITUTED LAV SPIKED FACTOR IX ($10^{5.25}$/ML)	REDRIED	NONE	$10^{5.25}$/ML
	B.	RECONSTITUTED LAV SPIKED FACTOR IX ($10^{5.25}$/ML)	REDRIED	60° C/ 24 HRS.	UNDETECTABLE*

*LESS THAN 10 VIABLE VIRUS PARTICLES PER ML.

FF/cb
12/3/84

HEAT TREATED PROTHAR:

REDUCTION IN INFECTIVITY OF MODEL VIRUSES

LABORATORY WORK CARRIED OUT BY:

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REVIEWED AND APPROVED BY:

GRO-C
SUDHISH CHANDRA, Ph.D. — 12.10.84 Date

GRO-C
CRISS TARR, Sc.D — 12/4/84 Date

GRO-C
FRED FELDMAN, Ph.D. — 12/6/84 Date

HEAT TREATED PROTHAR:
REDUCTION IN INFECTIVITY OF MODEL VIRUSES

SUMMARY

A study was designed to explore whether the heat treatment of Prothar would result in reduction of infectivity associated with enveloped viruses which are models for human blood-borne infectious agents. Sindbis, Vesicular Stomatitis (VSV) and Pseudorabies were used as model viruses. Heat treatment of Prothar at 60° C for 30 hours resulted in a reduction of infectivity by at least 7.74 logs, 4.95 logs, and 3.81 logs for Sindbis, VSV, and Pseudorabies viruses respectively.

HEAT TREATED PROTHAR:
REDUCTION IN INFECTIVITY OF MODEL VIRUSES

INTRODUCTION

It has been known for a long time that Hemophilia B patients treated with Factor IX concentrates are at risk of exposure to blood-borne infectious agents, mainly Hepatitis B virus (1-7) and the agent(s) of Non-A Non-B Hepatitis (8). More recently the possible transmission of Acquired Immune Deficiency Syndrome (AIDS) in hemophilia patients has also been postulated (9). Efforts are being made to produce a Factor IX preparation free of virus infectivity. Exposure of Factor IX preparations to sufficiently controlled heat treatment may result in inactivation of known viral agents. However, animal models for detection of infectivity of the above viruses are cumbersome, insensitive, and very expensive. A reasonable alternative is to study the inactivation of model blood-borne viral agents for which sensitive and rapid assay methods are available. Viruses like Sindbis, Pseudorabies, and Vesicular Stomatitis (VSV) are enveloped viruses having a lipid coat, and were considered models to satisfy these conditions.

MATERIALS AND METHODS

A. Viruses

Sindbis virus (Strain Ar-339) and VSV (Indiana Strain) were obtained from the American Type Culture Collection.

Both stock viruses were propagated in chick embryo fibroblasts (CEF). Pseudorabies virus (Strain Aujeszki) was also obtained from the American Type Culture Collection and propagated in vero cells.

B. Factor IX Preparation

Factor IX used in this study was Prothar manufactured by Armour Pharmaceutical Company.

C. Virus Assays (Plaque Assays)

All three viruses were assayed by a method similar to that described by Pfefferkon and Hunter (10). Sindbis and VSV were assayed using CEF cells, whereas vero cells were used for Pseudorabies. A description of Sindbis virus assay is as follows: CEF were seeded into 100mm plastic dishes and allowed to grow to confluence (48 hours). Serial tenfold dilutions of samples were made in minimal essential medium (MEM) containing 10% fetal calf serum and 1.0 ml inoculated onto replicate plates from which culture medium had previously been drained. The plates were incubated for one hour at 37° C in 5% CO_2 after which the inoculum was aspirated and 10 ml per plate of overlay medium added. The overlay medium consisted of MEM with 10% heat inactivated fetal calf serum and 1% agarose. The overlay was allowed to solidify after which cultures were incubated at 37° C in 5% CO_2 for about 24 hours. At this time, cytopathology was

evident in positive cultures. A 0.002% solution of neutral red in phosphate buffered saline was then added, and cultures were incubated an additional four hours. After this period, plaques were counted.

D. Heat Treatment

Lyophilized preparations were allowed to stand in a waterbath set at 60° C for 30 hours, after which the vials were removed and stored at -70° C until assayed.

RESULTS

Several vials of Prothar were reconstituted, pooled, and spiked with one virus strain at a time. After mixing, the bulk was divided, new vials were then filled, freeze dried, and heated. Virus infectivity was assayed in the heated vials and control non-heated vials to ascertain the reduction in virus infectivity by heat treatment. Table I shows the effect of heat treatment on three viruses. Reductions of at least 3.8 logs, 4.95 logs, and 7.74 logs were observed for Pseudorabies, VSV, and Sindbis viruses respectively.

TABLE I

EFFECT OF HEAT TREATMENT ON MODEL VIRUSES ADDED TO PROTHAR

VIRUS	VIRUS INFECTIVITY			
	INITIAL PFU/ml	HEAT TREATED PFU/ml	REDUCTION	
			FOLD	IN LOG
ENCEPHALOMYOCARDITIS	5.45×10^7	< 1	$> 5.45 \times 10^7$	> 7.74
[illegible]	2.22×10^4	< 0.25	$> 8.88 \times 10^4$	> 4.95
PSEUDORABIES	1.60×10^3	< 0.25	$> 6.40 \times 10^3$	> 3.81

[illegible]/cd
[illegible]/7/84

AP000431

REFERENCES

1. Boklan, B.F. Ann Intern Med. (1971); 74, 298

2. Faria, R. and Fiumara, N.J. New England J. Med. (1972); 287, 358-359

3. Hellerstein, L.J. and Deykin, D. New England J. Med. (1971); 284, 1039-1040

4. Kasper, C.K. and Kipnis, S.A. J. Am. Med. Ass. (1972); 221, 510

5. Iwarson, S.; Kjellman, H. and Teger-Nilsson, A.C. Vox Sang (1976); 31, 136-140

6. Oken, M.M.; Hootkin, L and Jager, R.L. de Dig. Dis. Sci. (1972); 17, 271-274

7. Sandler, S.G.; Rath, C.E.; Wiekerhauser, M.; Dodd, R.Y. and Greenwalt, T.J. Transfusion (1973); 13, 221-223

8. Wyke, R.J.; Thronton, A; Portmann, B.; Zuckerman, A.J.; Tsiquaye, K.N.; White, Y.; Das, P.K. and Williams, R. Lancet (1979)i, 520-524

9. Jaffe, H.W.; Bregman, D.J. and Selik, R.M. J. Infec. Dis. (1983); 148, 339-345

10. Pfefferkorn, E.R. and Hunter, H.S. Virology (1963); 20, 433-445

7. STUDIES IN HUMANS

Heat treated Factorate proposed for introduction onto the U.K. market has been and is currently on the U.S. and German markets. Hence there is already considerable exposure of patients to this product. Furthermore, the product is being used in the U.K. on a 'named patient' basis and has been used in the abortive clinical study (CTX 0231/0070A). Additionally a half-life study has been conducted in the U.K. by Dr. Rizza, Consultant Haematologist at the Oxford Haemophilia Centre. The heat treated product is clinically effective and the product appears to have a similar safety profile.

Two half-life studies have been conducted. The first was carried out in the U.S.A. with Factorate containing hepatitis immune globulin which has been heat treated as proposed in this application.

Six male patients, four with severe and two with moderate Haemophilia A were given the heat-treated material in a cross-over study against unheated material. Blood sampling was carried out at 0, 15, 30 and 60 minutes and 2, 4, 6, 8, 24 and 48 hours after injection and patients were subjected to general clinical assessment at 0 and 15 minutes and 1, 2 and 4 hours. Circulating levels of Factor VIII activity were calculated from the biological response in tests of coagulation function. The results indicated absence of adverse reactions or local intolerance to infusions. No clinically significant effects of measured parameters were detected as a result of the treatments. Evaluation of the half-life characteristics of the two preparations showed that these were practically identical. The biological half-life values (i.e. elimination half-lives) of the two preparations were calculated to be 10.88 ± 4.11 hours for heat-treated Factorate product and 10.88 ± 3.57 hours for the unheated Factorate product. Recovery values calculated using the formula:

$$\text{Recovery (K)} = \frac{\text{kg body weight x Factor VIII rise (u/dl)}}{\text{dose of Factor VIII administered (u)}}$$

were 1.8 ± 0.2 for heated material and 1.9 ± 0.2 for unheated material.

The full report of this study is presented in the Appendix to this file, page 91.

The second study, as mentioned above was conducted by Dr. C. Rizza, Oxford Haemophilia Centre on the material used under the CTX 0231/0070A. The comparative study was conducted in four severe haemophiliac patients and indicates that the half-lives in second phase for both heat treated and conventional Factorate were essentially similar. Other clinical and laboratory parameters were unchanged between treatments. The report of this study is presented in the Appendix to this file, page 163.

ARMOUR001429

8. APPENDIX

CONTENTS (REPORTS) PAGE NO.

CONFIDENTIAL AND PROPRIETARY
NOT TO BE DUPLICATED

PROJECT: Factorate

STUDY NO.: PFR-82-050

TITLE

The effect of heat treatment on thrombin activation of Factorate.

OBJECTIVE: To determine whether conditions that may be suitable for pasteurization of Factorate affect the characteristics of its activation by thrombin.

SUBMITTED BY: G. Amphlett, Ph.D. 7/14/82

REVIEWED BY: GRO-C 7/14/82

__/__/__

PLASMA FRACTIONS DIVISION
PROTEIN BIOCHEMISTRY DEPARTMENT

AP000435

Background:

It has been known for some time that Anti-Hemophilic Factor (AHF) shows a pronounced increase in activity, as measured by a one stage coagulation assay, after a short preincubation with trace amounts of thrombin. Since highly purified preparations of AHF are only now beginning to become available, the mechanism of this activation is unknown: for instance, thrombin could form a stoicheometric complex with AHF or it could activate the protein by catalytic cleavage. It is likely, however, that thrombin activation has a physiological role: during normal coagulation the small amounts of thrombin generated initially would activate large amounts of AHF, causing production of still larger amounts of thrombin via the intrinsic coagulation cascade; this could therefore be an important amplification mechanism to allow rapid hemostasis. Regardless of any hypothetical mechanism, the ability of AHF to become activated by thrombin is widely accepted as characteristic of the native AHF complex as found in plasma. In any proposed pasteurization procedure for AHF it is important to ensure not only that AHF activity, measured by a standard one or two stage clotting assay, is preserved as far as possible, but also that the extent of activation by thrombin should be changed as little as possible.

This report summarizes data contained in a preliminary report from Dr. Marx to Drs. Landaburu and Arinda dated December 29, 1980.

Experimental Approach:

Factorate (Armour Lot T55410) was heated in the dried state at 70° for 25 hr. and reconstituted with either water for injection (WFI) or WFI containing 0.25 M $CaCl_2$. AHF was activated by preincubation with purified human thrombin (5 ng/ml final concentration) for up to 30 min. then assayed in a standard one stage PTT assay. Control experiments showed that the amount of thrombin used for activation had no significant effect on the subsequent assay.

Results and Discussion:

Figure 1 shows the time course of activation of AHF by thrombin. A peak of activity was reached after approximately 2-5 min., corresponding to a 12 fold increase over starting activity. With further preincubation with thrombin, less activation was observed until, after 15-20 min. the activity declined below initial levels. These results are in accord with published data.

Heated and control (unheated) samples of Factorate were preincubated with thrombin as above for 2 min., in order to ensure extensive activation. The activities of these samples, as measured by a one stage PTT assay before and after thrombin activation, are shown in Table I. There was no significant difference between the PTT levels of non-activated heated and unheated samples: in addition there was no difference in activities of samples reconstituted in WFI or in WFI containing 0.25 M $CaCl_2$. There was also very little difference in the extent of thrombin activation (10 to 16 fold) observed with these samples.

Thus the heat treatment described has little significant effect on either the non-activated or the thrombin activated AHF levels of Factorate.

GRO-C

G. Amphlett, Ph.D.

GA:kmc

FIGURE 1

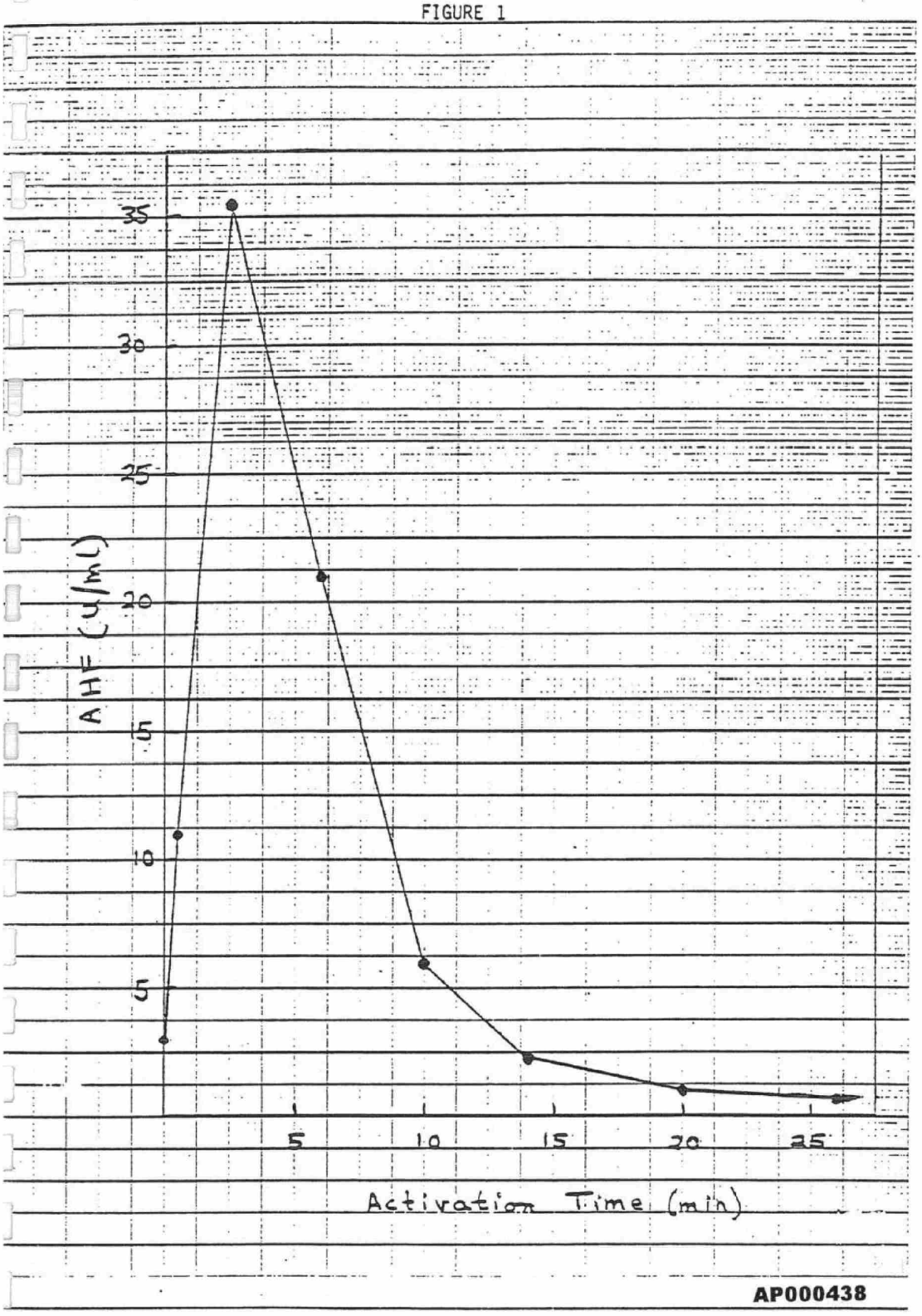

35
30
25
20
15
10
5
AHF (U/ML)
5
10
15
20
25
Activation Time (min)
AP000438

Table I

Effect of heating (70°/25 hr) on non-activated and thrombin activated AHF levels of Factorate.

	AHF Activity (Units/ml)		
Sample	Before Activation	After Thrombin Activation	X-Fold Activation
Unheated	7.0	99	14
Unheated + 0.25 M $CaCl_2$	6.4	105	16
Heated	7.2	75	10
Heated + 0.25 M $CaCl_2$	6.6	81	12

CONFIDENTIAL AND PROPRIETARY

NOT TO BE DUPLICATED

PROJECT: Factor VIII

STUDY NO.: PFR-82-043

TITLE

Effect of Heating on Factorate

OBJECTIVE: To heat Factorate in the dry state at 60°C for 24 hrs. and then immunochemically determine if the proteins have been altered by this treatment.

SUBMITTED BY: R. E. Branson, Ph.D
R. D'Alisa, Ph.D. 6/29/82

REVIEWED BY: GRO-C 6/30/82

_____ __/__/__

PLASMA FRACTIONS DIVISION
PROTEIN BIOCHEMISTRY DEPARTMENT

17-1

AP000440

Background:

The ability to pasteurize Factorate would be of great advantage in that it would provide a hepatitis-safe product. However, heating a complex protein mixture for a lengthy period of time might give rise to alterations in some or all the proteins. These changes might manifest themselves as neoantigens whose presence might be detected by immunochemical means.

Experimental Approach:

Antibody to Factorate, either unheated or heated in the dry state at 60°C for 24 hrs, was elicited in New Zealand white rabbits. The antibodies were then used in Ouchterlony gel diffusion and two dimensional immunoelectrophoretic techniques in an attempt to detect the presence of new antigens brought about by the heating procedure.

Results:

I. Two dimensional IEP analysis.

Antibodies elicited by heated and unheated Factorate were run against both heated and unheated antigens. Although a number of animals were immunized with each preparation, each antiserum was run individually, rather than as a pool. The patterns obtained were essentially identical throughout. No new peaks were observed following heating of Factorate nor was the mobility of any of the peaks altered (Figure 1).

II. Ouchterlony gel diffusion analysis.

Gel diffusion was carried out with individual antisera to heated and unheated Factorate vs. heated and unheated antigen. In all instances, heated and unheated material gave lines of total identity. No spurs were observed (Figure 2).

Analysis was also carried out with antisera specific for various plasma proteins such as immunoglobulin, fibrinogen, albumin and transferrin. In this case too, heated and unheated Factorate gave lines of identity, indicating no alterations in these proteins (Figure 2).

Discussion and Conclusions:

The techniques currently available failed to show any significant differences between heated and unheated Factorate. The complexity of the patterns seen in two dimensional IEP make it difficult to state with certainty that no changes have occurred. Furthermore, some differences in pattern are observed from one individual antiserum to another, even though all the rabbits were immunized with the same immunogen. In these cases, it is difficult to determine if the differences are true alterations in the proteins being analyzed or simply variation of antigen immunogenicity from animal to animal.

GRO-C

R. D'Alisa, Ph.D.

GRO-C

R. E. Branson, Ph.D.

kmc

AP000442

Appendix of Technical Details:

I. Immunization of Rabbits

A. A vial of Factorate lot #TM223*was heated at 60°C for 24 hrs.
B. One vial each of heated or unheated Factorate was reconstituted with 12.5 ml sterile water lot #82001 and 12.5 ml complete Freund's adjuvant for a total of 25 ml/vial.
C. 3 New Zealand white rabbits (2.5 Kg.) were immunized with each of the preparations.
D. Each rabbit received 3 immunizations at monthly intervals, each immunization consisting of 4 ml emulsion containing 48.96 mg protein; immunization was subcutaneous along the back of the rabbit in several sites.
E. Rabbits were bled at 2 weeks, 10 weeks and 16 weeks and serum from the 10 week bleed was used for this study.

II. Crossed IEP

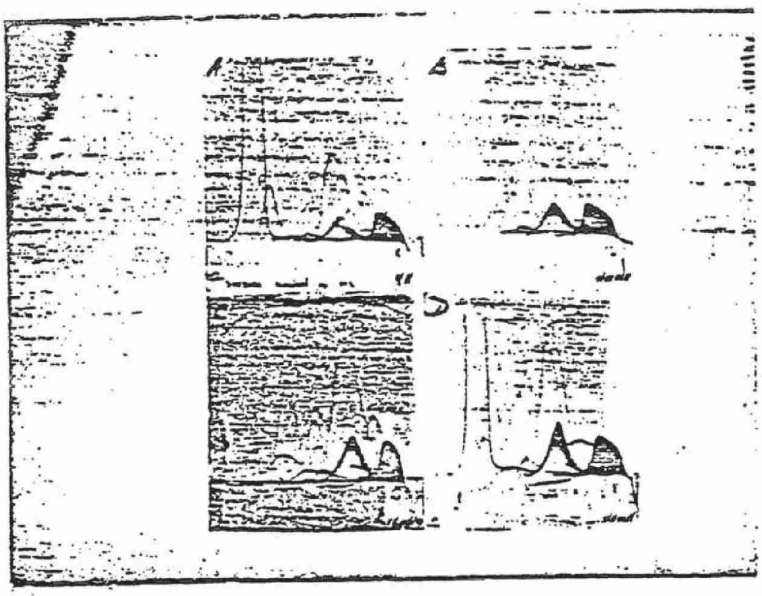
A. Two dimensional IEP was performed in Tris-barbiturate buffer, pH 8.6 as follows: 1) the first dimension was run at 10°C, 10 V/cm for 1 hr. using 5 ul of each sample to be analyzed, 2) the second dimension was run in 1% agarose containing 0.25 ml antiserum per 12 ml agarose, at 10°C for 18 hrs. at 2V/cm.

III. Ouchterlony Analysis

A. Gel diffusion was carried out in 1% agarose in Tris-barbiturate buffer, pH 8.6 for 18 hrs. at 4°C using 10 ul of antiserum and 5 ul of antigen.

* Final product lot number U22310

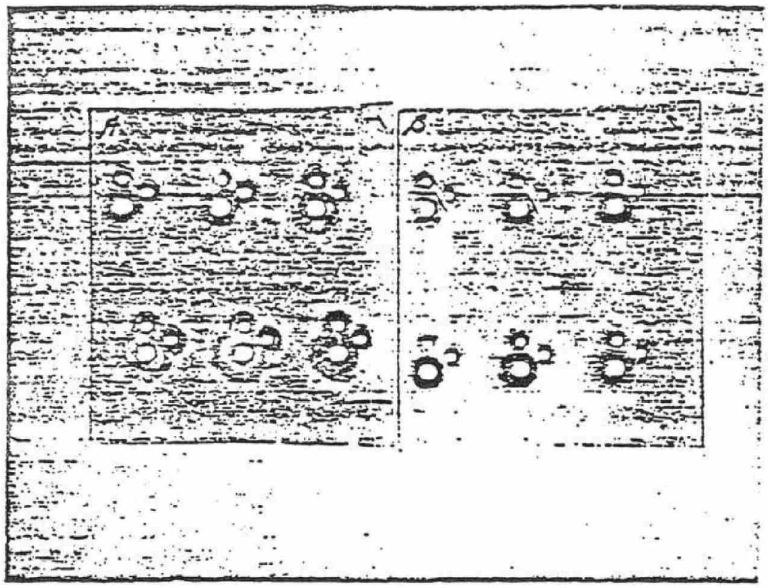
Figure I



Two Dimensional Immunoelectrophoresis

A. heated sample vs. antiserum to heated sample
B. heated sample vs. antiserum to non-heated sample
C. non-heated sample vs. antiserum to non-heated sample
D. non-heated sample vs. antiserum to heated sample

Figure II



Ouchterlony Gel Diffusion

Plate A top row - 3 individual antisera to heated material vs. heated and unheated antigen.

Plate A bottom row - 3 individual antisera to non-heated material vs. heated and unheated antigen.

Plate B top row, left to right - antisera to IgG, albumin and fibrinogen vs. heated and unheated samples.

Plate B bottom row, left to right - antiserum to α lipoprotein, B lipoprotein and transferrin vs. heated and non-heated samples.

PROJECT: Factor VIII-Hepatitis Attenuated

STUDY NO.: PFR83-006

TITLE

Effect of Heating on Proteins Present in Factorate

OBJECTIVE:

To use immunochemical analysis to determine if heating Factorate elicits neo-antigens.

SUBMITTED BY: GRO-C 7/11/83
J. Newman, Ph.D.

GRO-C 7/11/83
R.E. Branson, Ph.D.

REVIEWED BY: GRO-C 7/12/83
M.E. Hrinda, Ph.D.
PLASMA FRACTIONS DIVISION
PROTEIN BIOCHEMISTRY DEPARTMENT

AP000446

Background

The exposure of plasma proteins present in Factorate to temperatures sufficient to attenuate the infectivity of hepatitis virus may result in altering the structure of the proteins. These modified proteins may then act as neo-antigens when infused into patients. Comparison of antibodies produced in rabbits immunized with nonheated Factorate or a heated preparation containing added Hepatitis B antibody may provide a means of detecting the presence of neo-antigens by immunochemical methods.

Methodology

Experimental Approach:

I. Immunization of Rabbits

A. One vial of Factorate lot X22502 (nonheated) and one vial of Factorate lot K963110 with added Hepatitis B antibody (heated 60°, 30 hours) were reconstituted with 10 ml of sterile water for injection. Two ml of each lot of Factorate were homogenized with 2 ml of Freunds complete adjuvant. The remaining Factorate was aliquoted out into 1 and 2 ml fractions and frozen at -40°C until needed.

B. Two groups of 3 New Zealand Whites (2.5Kg) were used in this study. The animals were ear tagged and bled before receiving the first immunization. The rabbits' backs were shaved and each animal then received an initial injection of 1ml intradermally at several sites across the back. The animals were boosted at 2 weeks, 6 weeks and 9 weeks. Six days after the 6 week and 9 week boosts the animals were bled and the antiserum analyzed by two dimensional crossed immunoelectrophoresis.

II. Two Dimension Crossed Immunoelectrophoresis

The electrophoretic procedure used was that of Crowle and Miller (Journal of Immunological Methods vol. 43 15-28, 1981. The staining procedure was that of Crowle and Cline (Journal of Immunological Methods vol 17 379-381, 1977). The first dimension was run at 16 volts/cm for 1 hour at 4°C using 12 ul of a 1 to 3 dilution of the original Factorate material. The second dimension was run in a 1% agarose gel containing 0.75ml of antisera obtained from the rabbits injected with the heated or unheated Factorate per 10ml agarose. The electrophoresis was carried out at 4°C and 16 volts/cm for 2 hours 25 minutes.

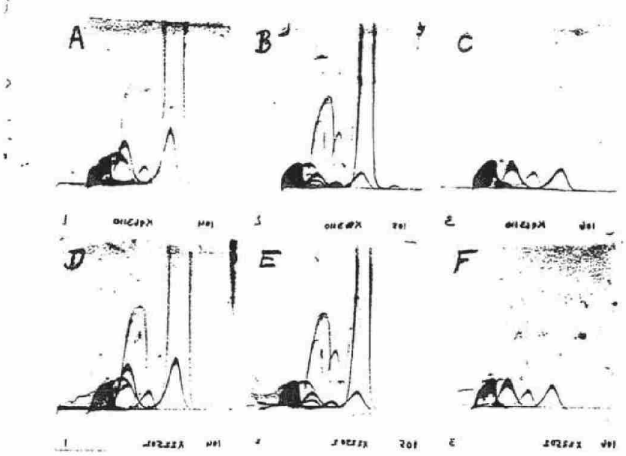
Results

A comparison by crossed immunoelectrophoresis of heated and nonheated Factorate using antibodies against heated Factorate is shown in Figure 1. Although there are peaks present in some gels that are absent in others, there is no evidence of a new peak consistent in all of the gels. Data obtained from electrophoresis runs using antibodies raised against the nonheated Factorate show this variability which indicates the inherent differences among animals in their responsiveness, to the same antigen.

Discussion and Conclusion

The variation in immunological responsiveness of the individual rabbits to the injection of the same immunogenic material (i.e. either preparation of Factorate) is as great as the variation between the group of rabbits receiving one or the other preparation. The variation in peak height to any one immunogen probably demonstrates the differences between rabbits and not differences due to the presence of neo-antigen. Since all of the rabbits can show consistency in responding to some of the proteins present in both preparation, then the lack of consistent response to the heated Factorate in the group of rabbits which received that material also suggests that no new antigenic site is present on any of the proteins that were capable of eliciting an antibody response. Therefore, these results suggest that heating Factorate with added Hepatitis B antibody does not cause changes in the protein's structure significant enough to elicit new antibodies.

Figure 1



Two Dimensional Immunoelectrophoresis

A. K963110 heated sample vs antisera 104 to heated sample

B. K963110 heated sample vs antisera 105 to heated sample

C. K963110 heated sample vs antisera 106 to heated sample

D. X22502 nonheated sample vs antisera 104 to heated sample

E. X22502 nonheated sample vs antisera 105 to heated sample

F. X22502 nonheated sample vs antisera 106 to heated sample

REPORT #: ED-82-08

DATE OF REPORT: July 29, 1982

"HALF-LIFE AND RECOVERY OF HEATED AND UNHEATED ANTIHEMOPHILIC FACTOR VIII IN HEMOPHILIC DOGS"

AUTHOR: GRO-C
John W. Dietrich, Ph.D.

This investigation was conducted by Dr. J. Dodds, Research Director, Laboratories for Veterinary Science, New York State Department of Health, Albany, NY.

DEPARTMENT OF ENDOCRINOLOGY
REVLON HEALTH CARE GROUP
DIVISION OF BIOLOGICAL RESEARCH AND DEVELOPMENT
ONE SCARSDALE ROAD
TUCKAHOE, NEW YORK 10707

SUMMARY

Human Antihemophilic Factor VIII (AHF) was infused into the cephalic vein in three dogs with hemophilia A; one canine received unheated AHF while two were treated with a preparation which was heated in the dry form at 60 C for 30 hours. The rate of infusion was 8 ml/min over a period of 3.5 - 4.0 minutes, with the total dose of AHF administered being 770 U. Fifteen minutes prior to the infusion the dogs were treated with an antihistamine (chlorpheniramine maleate, 7.5 mg). Blood samples were collected from the cephalic or saphenous veins immediately before and at the following times subsequent to AHF administration: 15, 60 and 90 minutes and 3, 5, 8 and 24 hours. The following parameters were quantitated from platelet-free-plasma: factor VIII:C (coagulant activity) and factor VIII:RA (von Willebrand factor; related antigen activity). Total protein, hematocrit, white blood cells (WBC) and platelets were quantitated from citrated whole blood. Respiration rate, pulse and rectal temperature were also monitored during the course of the study.

Factor VIII:C was increased by 0.29 U/ml (76%) 15 minutes after infusion of unheated AHF. The percent recovery of exogenous AHF in this dog was 65%. The half-life of disappearance had to be calculated between 3 and 8 hours, since the 24 hour time-point had decreased to less than baseline; the approximate half-life was eight hours (the normal half-life in man is 8-12 hours). Infusion of heated AHF elevated FVIII:C by 0.47 and 0.49 U/ml (134% and 258%) at 15 minutes in the two dogs treated with this preparation. The recovery of AHF in these animals was 81% and

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97%, with the half-lives being 22 and 18 hours, respectively.

Infusion of unheated AHF resulted in a maximum increase in FVIII:RA of 0.41 U/ml (26%) above baseline; this elevation was observed at the earliest time point tested (15 minutes). After 24 hours plasma FVIII:RA had decreased to below baseline in this animal. Infusion of heated AHF was associated with maximal increases in FVIII:RA of 0.45 and 0.69 U/ml (46% and 64%) in dogs A and B; these levels were attained at 15 and 90 minutes, respectively, after the infusion of AHF. After 24 hours plasma FVIII:RA was still elevated by 24% in dog B while in dog A the level had returned to baseline.

There was little effect of either heated or unheated AHF on hematocrit or total protein; minimal changes were observed in all three dogs. Both preparations were associated with wide fluctuations in WBC and platelets during the 24 hours subsequent to the infusion. These changes were not, however, considered drug-related. One dog infused with heated AHF experienced wide fluctuations in pulse pressure during the subsequent 24 hours; other than this no drug-related effects on the other physiological parameters measured were observed.

INTRODUCTION

Hemophilia A is a congenital, sex-linked clotting disorder caused by a deficiency in the coagulant activity of antihemophilic factor VIII (AHF). This plasma factor is normally present as part of a complex of

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two proteins with differing activities; Factor VIII:C (FVIII:C, coagulant activity), which corrects the coagulation defect in patients with hemophilia A, and Factor VIII:RA (FVIII:RA, von Willebrand factor; related antigen activity), which is related to platelet aggregation and is reduced or absent in patients with von Willebrand's disease.

The preferred treatment for patients with hemophilia A is plasma concentrates since these have a high FVIII content. In addition the FVIII concentration is known, thereby providing for more adequate control of bleeding. The main disadvantage of these preparations is the risk of transmitting hepatitis virus since they are manufactured from large plasma pools. While presently available preparations of AHF are required to be negative for hepatitis B surface antigen, currently available tests are not sensitive enough to detect all potentially infectious plasma samples and ,further, there is no assay for non A-non B hepatitis. Therefore there still exists the risk of infection upon repeated administration of AHF.

Various treatments have been, and are being, utilized in an attempt to decrease or eliminate the hepatitis content of plasma concentrates, including pasteurization and ultraviolet light. Revlon Health Care Group has utilized a heat treatment of 60°C for 30 hours to reduce the risk of hepatitis contamination of its AHF product. These preparations retain essentially all of the <u>in vitro</u> activity of the nonheated material, however it is not known what effect, if any, this heat treatment has on the effectiveness of infused AHF. Therefore the present studies were conducted to evaluate the half-life and recovery of exogenous heated and unheated AHF, and to compare the effects of these preparations on various other parameters in the hemophilic dog.

STUDY OBJECTIVES

The objectives of the present study were to evaluate the effects of heat treatment of AHF on the recovery and half-life of FVIII:C and plasma levels of FVIII:RA in hemophilic dogs. In addition a number of hemotological and physiological parameters were also monitored throughout the study to ascertain the possible presence of any modification of the various proteins present in these concentrates.

METHODS

Three male hemophilic dogs, weighing 27.3 - 36.4 kg, were used for this study. All had basal factor VIII:C (FVIII:C) levels of less than 0.4 U/ml, assayed in a system using human reagents, with 1 unit of FVIII being defined as the amount of coagulant activity present in 1 ml of normal human plasma when assayed in this system. Since the dog plasma FVIII:C is 3-4 times greater than that found in the human, these levels can be considered very low. Fifteen minutes prior to Antihemophilic Factor VIII (AHF) infusion the dogs were pretreated with 7.5 mg of chlorpheniramine maleate by subcutaneous injection.

Antihemophilic Factor (Human) Dried Factorate, Generation II (Armour Pharmaceutical Co., Kankakee, IL: Lot V28602), containing 770 U/vial of FVIII:C activity, was reconstituted with 30 ml of Sterile Water for Injection, USP (Armour Pharmaceutical Co., Lot U10409). Two preparations of AHF were used, one which was unheated and one which was heated in the lyophilized form at 60°C for 30 hours. These preparations were

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infused into the cephalic vein at a rate of 8 ml/min for a duration of 3.5-4.0 minutes; the total amount of AHF employed was 770 U/dog.

Blood samples were collected from alternate cephalic or saphenous veins prior to and at the following times subsequent to infusion of AHF: 15, 60 and 90 minutes and 3, 5, 8 and 24 hours. All samples were collected into a 3.8% citrate solution and were then divided into two aliquots, with the citrated whole blood portion being used for quantitation of hematocrit, total protein, white blood cells (WBC) and platelets. The second aliquot was centrifuged twice to yield platelet-free-plasma, which was used to quantitate FVIII:C and factor VIII:RA.

FVIII:C

Factor VIII:C was quantitated using a one-stage assay. A reference (in-house human standard), containing by definition 1 unit of AHF was serially diluted with a citrate-saline buffer 1:20 to 1:160. One hundred microliters of these dilutions or of the dogs plasma samples were added to human AHF-deficient plasma (0.1 ml) (congenital deficient human plasma) and a phospholipid with contained surface activator (0.1 ml) (Actin, Dade Diagnostics, Miami, FL). This mixture was incubated at 37°C for 5-7 minutes, after which 0.1 ml of 0.035 M $CaCl_2$ was added and the clotting time was determined using an MLA coagulation timer (Medical Laboratory Automation, Mt. Vernon, N.Y.).

FVIII:RA

Factor VIII:RA was quantitated by electroimmunoassay using rabbit anti-canine FVIII serum which was absorbed with 8% ethanol-precipitated canine plasma. Agarose was dissolved in a 2 mM calcium lactate buffer;

10 ml of this buffer solution, containing 1.2% of the absorbed anti-serum, was cast on 80 x 95 mm glass plates and placed overnight in a moist chamber at 4°C. Wells (3 mm) were punched in the agarose 25 mm from the end of the gel, and 6 ul samples of standard or platelet-free-plasma were added and electophoresed at 15 mA and 20 V/cm in a Gelman Delux Electrophoresis Chamber for 6 hours at 25°C using Gelman High Resolution Buffer (Tris barbital-Sodium barbital, pH 8.8) and filter paper wicks as the conducting medium. The gels were washed in 0.05 M phosphate-buffered saline, pH 7.4, for 24 hours, dried by warm air and stained with Coomassie Brilliant Blue.

The standard used was a pool of plasma from normal adult dogs. Four dilutions (1:1, 1:2, 1:4 and 1:8) were used, whereas two dilutions of the samples (1:2 and 1:4) were added. The final percent FVIII:RA was calculated by averaging the peak heights for each sample dilution as compared to the curve obtained for dilutions of the normal standard.

WHITE BLOOD CELLS

White blood cells were quantitated in a Neubauer hemocytometer. Whole blood was introduced into a Thoma pipet, followed by the addition of diluting fluid. After thorough mixing one drop was added to the hemocytometer and the number of WBC was counted in the corner squares of the chamber using a 10-fold magnification.

HEMATOCRIT

The hematocrit was quantitated using standard laboratory procedures. A sample of citrated whole blood was introduced into a microhematocrit tube. The tube was centrifuged for three minutes and the hematocrit was measured using a microhematocrit tube reader.

TOTAL PROTEIN

Total protein was determined using a Goldberg refractometer. A single drop of whole blood was placed on the prism of the refractometer; the protein content was quantitated directly from the refractometer scale.

PLATELETS

Platelet counts were quantitated in a Neubauer hemocytometer. Whole blood was introduced into a Thoma pipet, followed by addition of diluting fluid. After thorough mixing one drop was added to the hemocytometer and the number of platelets in the center squares of the chamber were counted using a 40-fold magnification.

All parameters were measured in duplicate and results are reported as the average of these duplicates.

PHYSIOLOGICAL PARAMETERS

Respiration rate and pulse were monitored manually, while rectal temperature was monitored using a rectal thermometer.

RESULTS

FVIII:C

The effects of infusion of AHF on plasma FVIII:C are presented in Table I.

Administration of unheated AHF elevated FVIII:C from a basal level of 0.38 U/ml to a maximum of 0.67 U/ml at 15 minutes, an increase of 0.29 U/ml (76%). This was followed by a slow decrease up to three hours, after which the FVIII:C remained stable up to eight hours; between 8 and 24 hours the level decreased and at the end of the study plasma FVIII:C was below baseline. The half-life of disappearance was calculated by using the 3, 5 and 8 hour time points, giving a half life of approximately 8 hours. The recovery of exogenous AHF in this dog was 65%.

Infusion of heated AHF to dog A resulted in a maximum increase in FVIII:C 15 minutes after administration; at this time the level had increased by 0.49 U/ml (258%) from a baseline value of 0.19 U/ml to 0.68 U/ml. Plasma FVIII:C slowly declined over the remainder of the study to 0.27 U/ml at 24 hours, a value which was .08 U/ml (42%) above baseline. In this dog the recovery of AHF was 97% and the half-life was approximately 18 hours.

Administration of heated AHF to dog B increased FVIII:C from 0.35 U/ml at time zero to 0.82 U/ml at 15 minutes, a level which was 0.47 U/ml (134%) above baseline. There was a rapid decrease in FVIII:C over the next 45 minutes, at which time the value was 0.68 U/ml. The plasma level remained stable up to and including the three hour time point, after which there was a slow decrease during the remainder of the study. At 24 hours plasma FVIII:C was still elevated by 0.12 U/ml (34%) above baseline. The recovery of AHF in this animal was 81%, with the half-life of disappearance of exogenous AHF being 22 hours.

FVIII:RA

The effects of infusion of AHF on plasma levels of FVIII:RA are shown in Table II.

Basal FVIII:RA in the dog treated with unheated AHF was 1.58 U/ml; 15 minutes after the infusion this was increased to 1.99 U/ml, or 0.41 U/ml (26%) above baseline. Plasma FVIII:RA decreased over the ensuing 75 minutes to 1.63 U/ml and then remained stable up to five hours. At eight hours FVIII:RA had decreased to 1.35 U/ml and at 24 hours was reduced further to 1.17 U/ml, 26% below baseline.

In dog A, basal FVIII:RA was 0.98 U/ml; 15 minutes after administration of heated AHF this value had increased by 0.45 U/ml (46%) to 1.43 U/ml. Plasma FVIII:RA remained at this level up to the five hour postinfusion sample, after which time it decreased to the pretreatment value at 24 hours.

Administration of heated AHF to dog B resulted in an increase in FVIII:RA from a baseline value of 1.07 U/ml to 1.62 U/ml, or 0.55 U/ml (51%), at 15 minutes. Plasma FVIII:RA continued to increase up to 90 minutes, when a value of 1.76 U/ml, or 0.69 U/ml (64%) above baseline, was attained. The plasma level decreased slowly after this time but was still elevated by 0.26 U/ml (24%) over the zero time value at the end of the study.

HEMATOCRIT

The effects of infusion of heated and unheated AHF on the hematocrit are presented in Table III. Both preparations caused a decrease in hematocrit which was evident as early as 15 minutes after the infusion. In the dog treated with unheated AHF the hematocrit was reduced up to 24 hours, whereas in the two dogs treated with the heated material the value had returned to baseline by the end of the study. The observed changes were not of great significance, as all values remained within the normal range (35%-53%) for dogs of this age.

TOTAL PROTEIN

Table IV illustrates the effects of AHF on total blood protein. Both preparations produced minor changes in protein. Unheated AHF caused an initial decrease, with the maximal change being 0.3 gm%

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one hour after administration. Protein then increased above baseline at five and eight hours and then returned to the preinfusion value at the end of the study. The heated AHF preparations also slightly decreased total protein, with the maximal reduction being 0.1 gm% in dog A and 0.4 gm% in dog B. These levels returned to baseline by 24 hours and, in the case of dog A, were increased by 0.5 gm% at the end of the study.

The normal range of total protein in dogs is 4.9-9.6 gm%, indicating that neither AHF preparation had a significant effect on this parameter over the 24 hour postinfusion period.

WHITE BLOOD CELLS

The effects of administration of AHF on WBC are shown in Table V. In all three dogs marked alterations in WBC in the 24 hours subsequent to AHF administration were observed. Unheated AHF was associated with an increase in the WBC count of 5060/mm^3 (33%) one hour after administration, which was followed by a reduction of 4840/mm^3 (31%) from baseline at three hours. The count slowly increased thereafter and by 24 hours was only 6% below the preinfusion level.

Both dogs treated with the heated AHF preparation demonstrated a rapid reduction in WBC of 60% (dog A) and 15% (dog B) at 15 minutes and 75% (A) and 41% (B) at one hour. The WBC count returned to the preinfusion level within the next two hours in both dogs, but this return was followed by a second reduction and subsequently a third at the end of the study. At 24 hours WBC were reduced by 78% (A) and 57% (B), compared to the zero time blood sample.

The normal range of WBC is 8300-19500/mm^3. While these data suggest that the heated AHF produced leukopenia, the investigator who conducted these studies did not feel that these reductions were significant and stated that such drastic fluctuations are not uncommon in hemophilic dogs.

PLATELETS

The effects of AHF on platelet counts are shown in Table VI.

Infusion of AHF was associated with a rapid increase in platelets; after 15 minutes this parameter had increased by 113% in the dog treated with unheated AHF and by 51% (dog A) and 8% (dog B) in the animals which received the heated material. In the dog infused with unheated AHF the platelet count returned to baseline after five hours but then increased again by 175% at the end of the study. In dog A platelets returned to baseline at one hour and then increased by 61% at 1.5 hours; this was followed by a second decrease and then another increase. At eight hours the platelet count was 83% above baseline, while at 24 hours the level had returned to near the basal level. In dog B, the platelet count rapidly decreased after the initial elevation and then increased again by 27% at five hours; subsequent to this, there was a 34% reduction at the end of the study. The normal platelet count in dogs is 200,000-600,000, again suggesting an effect of AHF; however, such drastic fluctuations suggest that the effects were not due to the drug but rather may have been due to the stress of the experiment.

PHYSIOLOGICAL PARAMETERS

The effects of AHF on respiration rate are shown in Table VII.

All three dogs demonstrated increased respiration at varying times during the infusion period; this stimulation occurred to the point that the animals were panting so that an exact rate could not be determined. At those time points at which the respiration rate could be calculated, it varied between 24 and 32 breaths per minute in the dog receiving the unheated AHF and between 36-44 (A) and 24-32(B) breaths per minute in the dogs which had received heated AHF. The normal range of respiration is 20-30, suggesting that all dogs demonstrated increased respiration, even prior to the infusion. This may have been due to the stress of the study and does not appear related to the blood product.

The effects of AHF on pulse pressure are shown in Table VIII.

In the dog receiving unheated AHF, pulse pressure increased from an initial value of 120 mmHg to 132 mmHg at one hour. This was followed by a return to baseline at 1.5 hours and another increase to 148 mmHg at five hours; by the end of the study pulse pressure had returned to near baseline.

Dog A responded to heated AHF with an initial decrease in pulse pressure from a baseline value of 116 mmHg to 104 at 15 minutes. Pressure returned to baseline between 1.5 and 3 hours and increased to 128 mmHg at five hours. By the end of the study pulse pressure was near baseline.

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In the second dog (B) receiving heated AHF pulse pressure decreased rapidly from 104 mmHg at baseline to 68 mmHg at 15 minutes; pressure then returned to baseline at 60 minutes. During the remaining 23 hours pulse pressure decreased to a minimum of 56 at three hours but returned to baseline at the end of the study. Since the normal range of pulse pressure is 105-150 mmHg, this dog demonstrated a significant response to the infusion of AHF.

The effects of AHF on rectal temperature are shown in Table IX.

None of the dogs responded to AHF with drastic changes in rectal temperature. At eight hours post-infusion in the dog receiving unheated AHF, rectal temperature had decreased by 1.4 degrees, which returned to baseline by the end of the study.

In dog A, rectal temperature increased by a maximum of 1.2 degrees at one hour; subsequent to this increase temperature decreased to 1.3 degrees below baseline at eight hours. By the end of the study rectal temperature was returning to the baseline value.

Similarly in dog B there was an initial increase in rectal temperature of 1.0 degree at one hour which decreased to baseline by three hours. At the end of the study rectal temperature was 0.8 degrees below baseline.

The normal range of rectal temperature is 100.0 - 102.0 ^{o}F.

DISCUSSION

The primary objective of the present study was to evaluate the effects of pasteurization of AHF on recovery and disappearance of FVIII:C. Since the plasma product is to be used in patients with hemophilia A, it was felt appropriate to use an animal model with a similar deficiency, and therefore canines with hemophilia were used for this study.

The half-life of transfused FVIII:C in the circulation of hemophilics is typically 12-14 hours, but can be as short as 8 hours and as long as 20 hours. In the present study the unheated AHF was associated with a half-life of eight hours, compared to 18 and 22 hours for the heated preparation. Despite the fact that heating appeared to prolong the half-life, all values are within or close to the reported range; further, due to the limited number of animals included in this study, it is not valid to conclude that pasteurization reduced the disappearance rate of FVIII:C. However it can be concluded that heating does not result in a shorter half-life.

The same is true for the recovery of FVIII:C. Typically more than 50% of exogenous AHF is recovered from plasma. In the present study heating appeared to increase recovery; the two dogs infused with heated AHF demonstrated 81% and 97% recovery, as compared to 65% for the unheated preparation. However, again because of the limited number of animals, it can be concluded only that pasteurization does not adversely affect the recovery of the AHF in hemophilic dogs.

As with coagulant activity pasteurization did not appear to decrease the recovery and half-life of FVIII:RA, although these latter parameters were not calculated. The maximal increase in FVIII:RA subsequent to infusion of unheated AHF was 26% above baseline, as compared to 46% and 64% for the heated preparation. The duration of the increase was also longer for the heated material; in this instance FVIII:RA was increased up to eight hours post-infusion in one dog, while in the second animal treated with heated AHF the level was still considerably above baseline at 24 hours. In contrast, in the dog treated with unheated AHF the plasma FVIII:RA had returned to the preinfusion level 5-8 hours after administration. From these results it appears that pasteurization of AHF does not decrease the recovery or increase the rate of disappearance of FVIII:RA

Neither heated or unheated AHF produced major changes in the hematocrit or total protein content of blood. However, both preparations were associated with marked fluctuations in WBC and platelet counts. This was especially true for the pasteurized material; in those animals which received the heated AHF the WBC count was decreased initially by 41-75%, returned to baseline and then decreased again. This response was not observed after administration of unheated AHF. Platelet counts also demonstrated marked variability in the 24 hour post-infusion period. According to the investigator who conducted these studies, such fluctuations are not uncommon when hemophilic or normal dogs are infused even with saline or balanced electrolyte solutions. The reason for these fluctuations in white cell and platelet counts in dogs undergoing a variable stress from repeated handling and blood sampling has been attributed to the constant ebb and flow of cells,

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especially leukocytes, moving into and out of the pulmonary circulation, the sequestration of cells in the liver and the contractility of the canine spleen (1). The stress-induced neutropenia can be rapid and transient, causing a shift of leukocytes from the marginal pool, resulting in a 2-3 fold change in the peripheral count. Therefore it is unlikely that the changes in WBC and platelet counts observed subsequent to infusion of AHF were related to drug administration but rather were probably caused by the stress of the study.

Likewise, the changes observed in respiration rate and pulse pressure did not appear to be drug-related. Respiration rate was elevated in all dogs, while pulse pressure was reduced drastically in one animal which received heated AHF. Again these were probably related to stress and not the particular agent under study.

One animal which was infused with unheated AHF (not discussed in this report) experienced an anaphylactoid reaction within two minutes of initiating the infusion, after receiving approximately 10 ml of material. This animal had received two vials of human CPD-collected cryoprecipitate (containing 391 U of Factor VIII/vial, each containing 2.4 g of protein) three weeks prior to the present infusion. It appears that the anaphylactoid reaction was a direct result then, of prior sensitization from the human CPD-cryoprecipitate given previously. A similar finding from a second exposure to human plasma proteins occurred in another unrelated hemophilic dog from the investigators colony and has also been reported by others (2). The anaphylactoid reaction in the present study was documented by evidence of disseminated intravascular collapse (collapse, severe thrombocytopenia, prolonged thrombin clotting time and the presence

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of high levels of fibrinogen/fibrin degradation products). By seven hours post-infusion the animals coagulation profile and platelet count had returned to preinfusion values but there was a marked leukocytosis characteristic of a severe physiological insult. None of the other dogs in this study demonstrated any such reaction.

CONCLUSION

The present studies have demonstrated that pasteurization of AHF does not adversely affect the recovery or half-life of FVIII:C, nor does it appear to reduce the recovery or increase the disappearance of FVIII:RA in hemophilic dogs. Neither heated nor unheated AHF altered the hematocrit or total blood protein. While there were marked fluctuations in WBC and platelet counts subsequent to AHF administration, these responses did not appear to be drug-related. There appeared to be little effect of heated or unheated AHF on respiration rate, pulse pressure or rectal temperature.

TABLE I

EFFECT OF AHF ON PLASMA FVIII:C IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	FVIII:C, U/ml UNHEATED AHF	HEATED AHF (DOG A)	HEATED AHF (DOG B)
0	0.38	0.19	0.35
0.25	0.67	0.68	0.82
1.0	0.63	0.63	0.68
1.5	0.53	0.52	0.68
3.0	0.45	0.43	0.64
5.0	0.43	0.38	0.56
8.0	0.43	0.32	0.55
24.0	0.33	0.27	0.47

TABLE II

EFFECT OF AHF ON PLASMA FVIII:RA IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	FVIII:RA, U/ml UNHEATED AHF	HEATED AHF (DOG A)	HEATED AHF (DOG B)
0	1.58	0.98	1.07
0.25	1.99	1.43	1.62
1.0	1.82	1.43	1.74
1.5	1.63	1.43	1.76
3.0	1.62	1.42	1.66
5.0	1.70	1.45	1.69
8.0	1.35	1.36	1.55
24.0	1.17	1.01	1.33

TABLE III

EFFECT OF AHF ON HEMATOCRIT IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	HEMATOCRIT (%) UNHEATED AHF	HEATED AHF (Dog A)	HEATED AHF (Dog B)
0	51	53	50
0.25	48	52	48
1.0	47	52	47
1.5	46	49	48
3.0	47	52	44
5.0	47	52	46
8.0	47	50	45
24.0	46	53	51

TABLE IV

EFFECT OF AHF ON TOTAL BLOOD PROTEIN IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	TOTAL PROTEIN (gm%) UNHEATED AHF	HEATED AHF (Dog A)	HEATED AHF (Dog B)
0	6.0	5.7	6.0
0.25	5.9	5.7	5.6
1.0	5.7	5.6	5.7
1.5	5.8	5.6	5.9
3.0	5.8	5.7	5.7
5.0	6.1	5.8	5.8
8.0	6.3	5.7	5.6
24.0	6.0	6.2	6.0

TABLE V

EFFECT OF AHF ON WBC IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	WBC (per mm^3) UNHEATED AHF	HEATED AHF (Dog A)	HEATED AHF (Dog B)
0	15,510	14,355	11,550
0.25	15,675	5,775	9,790
1.0	20,570	3,630	6,765
1.5	12,760	14,410	7,370
3.0	10,670	3,080	12,265
5.0	12,705	14,630	6,710
8.0	13,475	16,225	21,120
24.0	14,575	3,135	4,950

TABLE VI

EFFECT OF AHF ON PLATELETS IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	PLATELETS (per mm^3) UNHEATED AHF	HEATED AHF (Dog A)	HEATED AHF (Dog B)
0	93,000	141,000	194,000
0.25	198,000	213,000	209,000
1.0	123,000	120,000	111,000
1.5	120,000	227,000	110,000
3.0	110,000	154,000	103,000
5.0	96,000	179,000	247,000
8.0	259,000	258,000	217,000
24.0	256,000	121,000	128,000

TABLE VII

EFFECT OF AHF ON RESPIRATION RATE IN HEMOPHILIC DOGS

TIME (h) POST-TRFATMENT	UNHEATED AHF	RESPIRATION RATE, BREATHS/MINUTE HEATED AHF (DOG A)	HEATED AHF (DOG B)
0	32	36	PANT
0.25	PANT	PANT	PANT
1.0	32	PANT	PANT
1.5	32	PANT	PANT
3.0	PANT	PANT	PANT
5.0	29	36	30
8.(	24	44	24
24.0	PANT	36	32

TABLE VIII

EFFECT OF AHF ON PULSE PRESSURE IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	PULSE PRESSURE, mmHg		
	UNHEATED AHF	HEATED AHF (DOG A)	HEATED AHF (DOG B)
0	120	116	104
0.25	124	104	68
1.0	132	104	100
1.5	120	108	88
3.0	120	120	56
5.0	148	128	80
8.0	116	124	72
24.0	124	120	104

TABLE IX

EFFECT OF AHF ON RECTAL TEMPERATURE IN HEMOPHILIC DOGS

TIME (h) POST-TREATMENT	RECTAL TEMPERATURE, °F		
	UNHEATED AHF	HEATED AHF (DOG A)	HEATED AHF (DOG B)
0	102.2	102.6	102.6
0.25	102.4	102.9	103.1
1.0	102.4	103.8	103.6
1.5	102.2	103.3	103.6
3.0	102.2	102.0	102.4
5.0	101.2	102.0	102.4
8.0	100.8	101.3	102.0
24.0	102.0	101.8	101.8

REFERENCES

1. O.W. Schalm, Jain, N.C., Carroll, E.J., Veterinary Hematology, 3rd (ED), Lee and Febiger, Philadelphia, 485-487, 1975.

2. H.S. Kingdon and Hassell, M., Blood 58:868-72, 1981.

HALF-LIFE CALCULATION

The elimination rate constant (β) was determined from the slope of the terminal segment of the semilogarithmic plot of blood concentration versus time by the method of least squares best-fit. The biological half-life ($T_{\frac{1}{2}}$) was calculated from the equation:

$$T_{\frac{1}{2}} = \frac{0.693}{\beta}$$

Blood concentrations were calculated by subtracting the baseline concentration of AHF from the concentration of AHF at each subsequent time point.

For the unheated AHF (Fig. I) the 24 hour time point was below baseline and therefore an approximate half-life, using the values for 3, 5 and 8 hours, had to be calculated. For this dog, the elimination rate constant was 0.089, resulting in a half-life of 7.8 hours.

For dog A, which received heated AHF (Fig. II) the elimination rate constant was 0.0381, giving a half-life of disappearance of 18.2 hours. For dog B, which received heated AHF (Fig. III), the elimination rate constant was 0.0306, resulting in a half-life of 22.5 hours.

FIGURE I

DISAPPEARANCE OF AHF IN A HEMOPHILIC DOG INFUSED WITH UNHEATED AHF

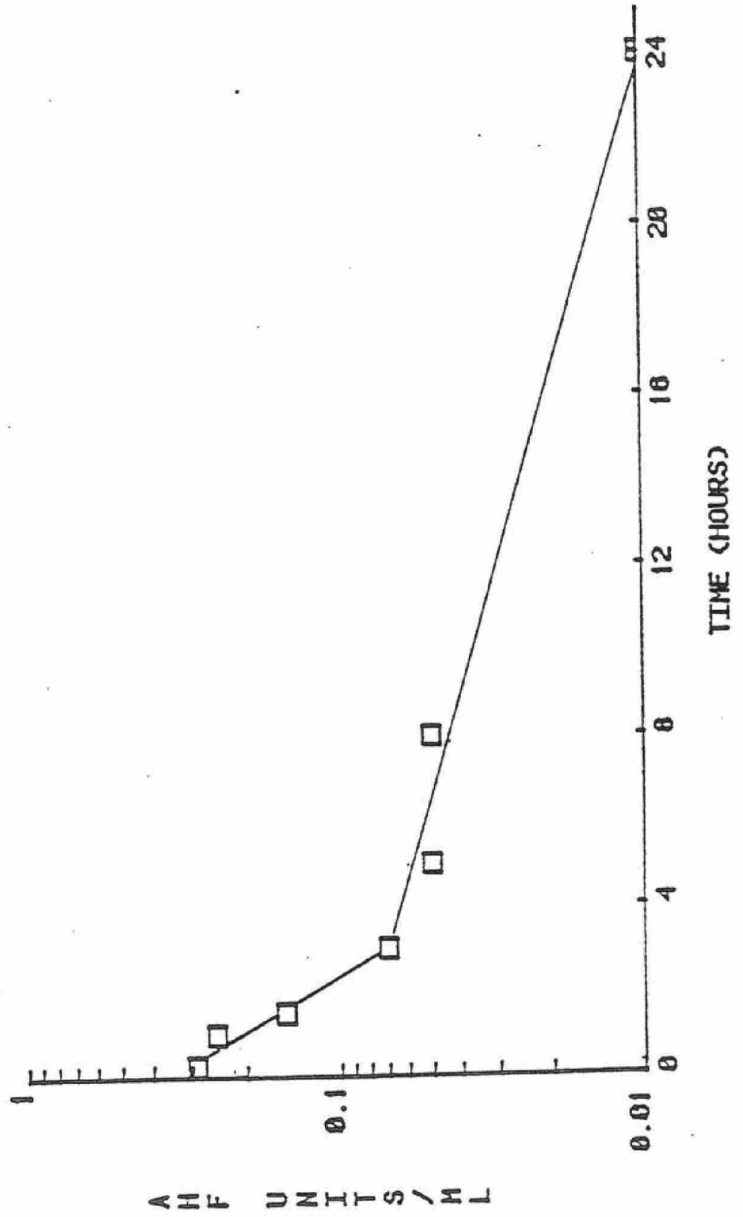
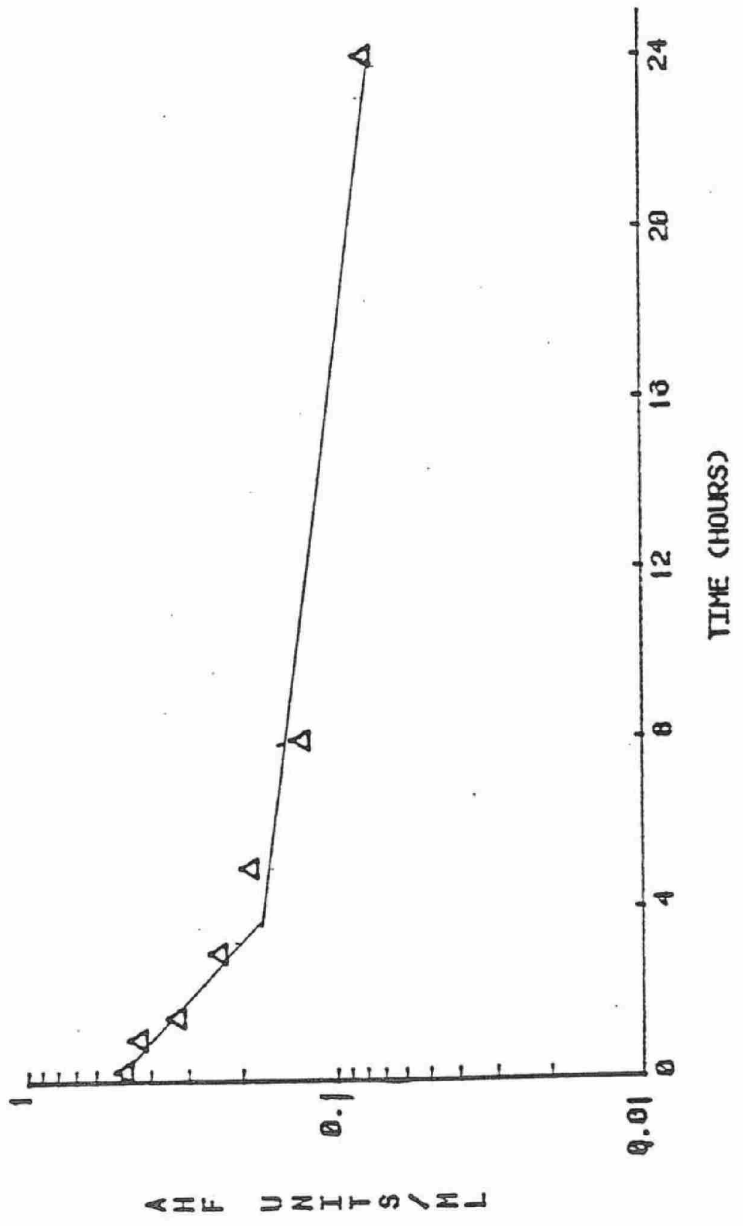


FIGURE II

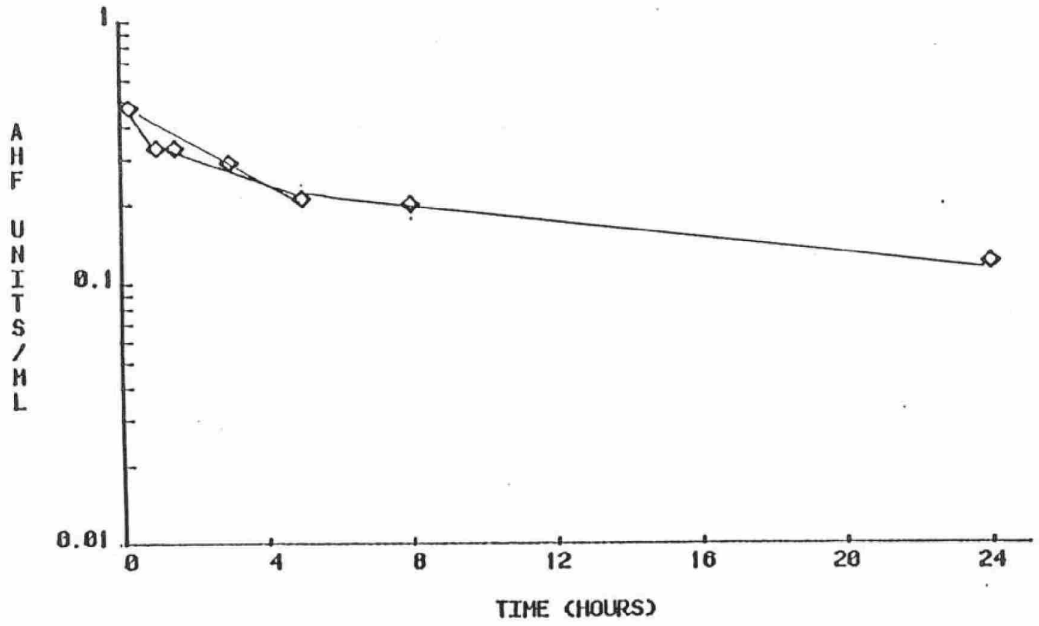
DISAPPEARANCE OF AHF IN A HEMOPHILIC DOG INFUSED WITH HEATED AHF (DOG A)



AP000480

FIGURE III

DISAPPEARANCE OF AHF IN A HEMOPHILIC DOG INFUSED WITH HEATED AHF(DOG 8)



REPORT #: ED-82-09

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"EFFECTS OF ANTIHEMOPHILIC FACTORATE, HEATED AND NONHEATED, ON HEART RATE AND MEAN ARTERIAL BLOOD PRESSURE IN ANESTHETIZED DOGS"

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SUMMARY

Antihemophilic Factorate (AHF) and diluent (Water for Injection, (WFI),USP) were infused into anesthetized dogs at a rate of 4.4 ml/min for approximately 4.5 minutes. The dose of AHF employed was 100 U/kg (230 U/min). Half of the dogs included in this study were treated with unheated AHF, while half were infused with material which had been pasteurized at 60 C for 24 hours. Heart rate (HR) and mean arterial blood pressure (MAP) were monitored throughout the study.

Infusion of WFI had little effect on HR in the majority of the animals tested; the maximum changes observed were a decrease of 15 beats per minute (bpm) in one dog and an increase of 20 bpm in another.

Administration of unheated AHF was associated with maximal increases of 14, 30 and 40 bpm in three dogs; in a fourth animal the HR was decreased by 5 bpm. Infusion of heated AHF reduced HR in two dogs during the administration period; the maximum changes observed in these animals were decreases of 16 and 20 bpm. In one dog HR increased by 20 bpm during heated AHF infusion, while in the remaining animal there was a minor increase of 6 bpm.

Administration of WFI was associated with a maximal increase in MAP of 13 mmHg in one dog; all other animals demonstrated negligible changes in MAP.

Infusion of unheated AHF had little effect on arterial pressure in three animals, with the changes ranging from a reduction of 7 mmHg to an

increase of 6 mmHg. One dog responded to the unheated preparation with an increase in MAP of 18 mmHg. Infusion of heated AHF increased MAP maximally by 22 mmHg in one dog. In the remaining three animals the increase in MAP ranged between 4 and 10 mmHg.

INTRODUCTION

Hemophilia A is a congenital sex-linked clotting disorder caused by a deficiency in the coagulant activity of antihemophilic factor VIII (AHF). Patients with this disorder are presently treated with plasma concentrates which have a high AHF content. The main disadvantage associated with these concentrates is the risk of transmitting hepatitis since they are manufactured from large plasma pools. Various treatments have been attempted to reduce or eliminate the hepatitis content of plasma concentrates, including pasteurization and ultraviolet light. Revlon Health Car Group has utilized a heat treatment of 60 C for 24-30 hours in an attempt to decrease the risk of hepatitis contamination of its lyophilized AHF product (Antihemophilic Factor (Human) Factorate^{R}, Armour Pharmaceutical Co.) While the heated preparation contains essentially all of the *in vitro* activity possessed by the unheated material, it is not known what effect, if any, this heat treatment has on the *in vivo* tolerance to AHF. Accordingly, the present study was conducted to examine the cardiovascular safety of heated and unheated AHF.

STUDY OBJECTIVE

The objective of the present study was to compare the effects of heated and unheated AHF on heart rate and mean arterial blood pressure in the anesthetized dog.

METHODS

Eight male beagles weighing 9.0-11.3 kg, which had been fasted overnight, were anesthetized with sodium pentothal (15 mg/kg) and α-chloralose (60 mg/kg); both anesthetics were administered slowly into the cephalic vein. At the beginning of each study the canine was intubated; all dogs were maintained on a heating pad (Gorman Rupp, Belleville, OH) for the duration of the study. During each study the lead II electrocardiogram, heart rate (HR) and mean arterial blood pressure (MAP) were continuously recorded on a grass Model 7B polygraph recorder (Grass Instruments, Quincy, MA).

Arterial pressure was recorded using a Statham P23ID pressure transducer (Oxnard, CA) connected to a PE 205 polyethylene cannula (Clay Adams, Parsippany, NJ) surgically implanted in the right femoral artery. The cannula was filled with heparin (Heparin Sodium Solution, ICN Pharmaceuticals, Cleveland, OH, Lot 8154), 200 U/ml. A second channel on the polygraph was utilized as an interface to convert to mean arterial pressure.

The lead II electrocardiogram was recorded with subdermal electrodes (Grass Instruments) placed on the right arm and left leg of each dog. Heart rate was monitored by a tachometer which was triggered by the output of the electrocardiogram.

PROTOCOL

Antihemophilic Factor (Human) Dried Factorate (Armour Pharmaceutical Co., Kankakee, IL, Lot TM223)* (AHF), containing 1245 U/vial, was reconstituted with Sterile Water For Injection (WFI), USP (Armour Pharmaceutical Co., Lot 8209 or Abbott Laboratories, Chicago, IL Lot 23-468-DK). The concentration of AHF after reconstitution was 52 U/ml. Half of the animals in this study received material which had been heated in the lyophilized form at 60 C for 24 hours, while the other half were treated with unheated AHF.

Test preparations were administered by constant infusion (Model 355, Sage Instruments, Cambridge, MA) into the left femoral vein through a polyethylene cannula (PE 190, Clay Adams) which was inserted prior to the initiation of each study. The rate of infusion of WFI and AHF was approximately 4.4 ml/min for a duration of 4.5 minutes. The total dose of AHF employed was 100 U/kg (approximate rate of infusion of 230 U/min).

Prior to the administration of WFI or AHF, all dogs were infused with normal saline (Mallinckrodt, Paris, KY, Lot KJST) at a rate of 0.014 ml/min for 20-40 minutes. After this initial stabilization period, WFI was infused at the rate described above. Subsequent to WFI saline was again administered at a rate of 0.014 ml/min (for 30-40 minutes) and this was followed by AHF infusion. Saline was also infused from the termination of AHF administration to the end of the study.

* Final product lot number U22310

RESULTS

The effects of infusion of unheated AHF on HR and MAP are illustrated in Table I.

Administration of WFI prior to infusion of unheated AHF decreased HR in three dogs by 5, 15 and 6 beats per minute (bpm) (3%, 9% and 4%, respectively) while in a fourth animal HR was increased by 20 bpm (18%) during the infusion. In all four dogs HR was stable prior to initiation of AHF treatment.

Infusion of unheated AHF produced tachycardia in three dogs, with the initial changes being observed 1-2 minutes after starting the infusion. The maximum elevation observed in these animals was 40 (28%), 30 (27%) and 14 (10%) bpm in dogs 2, 3 and 4, respectively. The HR returned to baseline by the end of AHF administration in dogs 2 and 4, and in dog 3 was at the preinfusion value within 10 minutes after the termination of AHF treatment. Dog 1 responded to unheated AHF with a decrease in HR of 5 bpm (3%); the rate decreased an additional 5 bpm during the subsequent saline infusion and returned to baseline by the end of the study, 30 minutes after the end of AHF administration.

Infusion of WFI produced negligible changes in MAP; pressure was increased maximally by 1-5 mmHg during the infusion period. Treatment with unheated AHF produced a significant change in MAP in one dog. In this animal (dog 3) MAP was increased initially at two minutes; the maximal change, an 18 mmHg (13%) rise, was observed near the end of the

(cont'd)

treatment period. Mean arterial pressure returned to near baseline in this animal six minutes after the end of the AHF infusion and was at baseline by the end of the study, 30 minutes after the termination of AHF. The other three dogs infused with the unheated preparation responded with maximal changes in MAP ranging from a decrease of 7 mmHg (5%; dog 1) to an increase of 6 mmHg (4%; dog 4).

The effects of administration of heated AHF on HR and MAP are shown in Table II.

Administration of WFI prior to pasteurized AHF had minor effects on HR. In two dogs HR was reduced maximally by 5 and 11 bpm (6% and 8%) at the end of the infusion, while in a third animal the rate increased by 3 bpm (2%). In the fourth canine HR was not affected by WFI.

Infusion of heated AHF increased HR in two dogs and produced bradycardia in the other two. The increased HR was observed within the first minute of the infusion in dog 3, at which time the rate was elevated by 20 bpm (17%); HR decreased during the remainder of the AHF infusion and was near baseline one minute after the termination of AHF treatment. The second animal which responded with an elevated HR (dog 4) demonstrated a maximum increase of only 6 bpm (5%) at the end of the infusion period.

Dog 1 responded to pasteurized AHF with a reduction in HR which was observed as early as one minute into the infusion; the maximum decrease of 20 bpm (18%) occurred at two minutes. Heart rate then increased during the remainder of the AHF treatment period and was at baseline one

minute after the termination of the infusion. The initial decrease in HR in dog 2 was observed three minutes into the infusion, while the maximum decrease, a reduction of 16 bpm (14%), was observed at the end of the AHF treatment period. Heart rate returned to baseline within two minutes of the termination of AHF administration.

Infusion of WFI increased MAP by 13 mmHg (10%) in dog 1, while in the remaining three dogs pressure was unaffected by WFI.

Administration of heated AHF elevated MAP in all four dogs tested, although a significant increase was seen only in dog 2. In this animal the initial increase in MAP was observed as early as one minute into the infusion, with the maximal change, 22 mmHg (17%), occurring at the end of AHF administration. Pressure returned to baseline in this dog within two minutes after the termination of the AHF infusion. In the remaining three dogs MAP was increased maximally by 4, 7 and 10 mmHg (3%, 5% and 7%, respectively) during the infusion; in all three animals the pressure returned to baseline by the end of the AHF treatment period.

DISCUSSION

The present study was conducted to compare the effects of heated (60 C, 24 hours) and unheated AHF on HR and MAP in anesthetized dogs. The diluent for this material, WFI, had little effect on either parameter investigated. A total of eight animals were infused with WFI; the maximal changes in HR observed in these dogs ranged from a decrease of 15 bpm to an increase of 20 bpm; most animals responded with changes of 5-10 bpm. Similarly there was little effect of WFI on MAP; the greatest change observed was an increase of 13 mmHg in one dog. The remaining

animals responded with changes of 0-5 mmHg.

Unheated AHF increased HR by 14-40 bpm in three dogs, while a fourth animal responded to AHF with a decrease of 5 bpm in HR. Two animals demonstrated an increase in HR during infusion with heated AHF; the maximum changes in HR observed in these dogs were 6 and 20 bpm. Two other dogs treated with pasteurized AHF demonstrated reductions in HR of 16 and 20 bpm. All of these changes were observed during the infusion period and most were evident as early as one minute after initiation of the infusion. Further, the responses were transient, in that HR had returned to baseline within 10 minutes of the termination of the infusion in most animals and, in some cases had returned to the basal level within one minute after the end of the AHF administration period. With respect to MAP the unheated preparation caused a significant effect in only one animal, producing an increase in pressure of 18 mmHg. In the remaining three animal treated with unheated AHF, MAP changes were minor. Similarly, one dog infused with heated AHF responded with an increase in MAP of 22 mmHg, whereas the maximum increase observed in the remaining dogs was 10 mmHg. As with the changes in HR, the effects on MAP occurred rapidly, within 1-2 minutes after initiation of the infusion and were very transient.

These results suggest that AHF, at a dose of 100 U/kg, has minor effects on HR and MAP in the anesthetized dog. Any changes which are seen in these parameters occur rapidly and are transient in nature. Further, pasteurization of AHF at 60 C for 24 hours does not appear to alter the reactivity of this clotting factor with respect to effects on HR or MAP.

CONCLUSION

Infusion of AHF, 100 U/kg, to anesthetized dogs produced minor changes in HR and MAP. In those cases where significant alterations did occur, they were observed soon after initiation of AHF administration and were transient in nature. Pasteurization of this material at 60 C for 24 hours did not increase the incidence of AHF-induced alterations in HR or MAP.

TABLE I

EFFECT OF UNHEATED AHF ON HR AND MAP IN ANESTHETIZED DOGS

TREATMENT	MAXIMUM CHANGE, ABSOLUTE (%)	
	HR	MAP
WFI		
Dog 1	-5 (3%)	---
2	-15 (9)	+5 (3%)
3	+20 (18)	+1 (1)
4	-6 (4)	+3 (2)
AHF		
Dog 1	-5 (3%)	-7 (5%)
2	+40 (28)	+3 (2)
3	+30 (27)	+18 (13)
4	+14 (10)	+6 (4)

TABLE II

EFFECT OF HEATED AHF ON HR AND MAP IN ANESTHETIZED DOGS

TREATMENT	MAXIMUM CHANGE ABSOLUTE (%) HR	MAP
WFI		
Dog 1	-5 (6%)	+13 (10%)
2	--	--
3	-11 (8)	+1 (1)
4	+3 (2)	+3 (2)
AHF		
Dog 1	-20 (18%)	+4 (3%)
2	-16 (14)	+22 (17)
3	+20 (17)	+7 (5)
4	+6 (5)	+10 (7)

DIVISION	PRODUCT OR PROJECT NAME
DEVELOPMENTAL THERAPEUTICS	FACTOR VIII, H.S.
DEPARTMENT	PROTOCOL NUMBER
CLINICAL PHARMACOLOGY	BB 1826-01
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APRIL 15, 1983	Susan Tannenbaum, M.D.

AUTHOR(S)

Susan Tannenbaum, M.D.

TITLE:

COMPARISON OF SAFETY AND HALF-LIFE OF SPECIALLY-PROCESSED FACTOR VIII WITH UNTREATED FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

EXTRAMURAL INVESTIGATOR(S)

Peter H. Levine, M.D., Principal Investigator
Doreen B. Brettler, M.D., Co-investigator
Worcester, Mass.

ABSTRACT:

Four patients with severe Hemophilia A and two with moderate Hemophilia A were treated with one infusion each of Factor VIII Concentrate, Untreated, and Factor VIII, Specially Processed, in an open 2-way crossover trial. Results show that the half-life and recovery of each product are equivalent.

No untoward reactions were experienced by the six patients during this trial.

RHC NUMBER AND KEYWORDS

Factor VIII Concentrate
Hepatitis Attenuated
Hemophilia A
Crossover Trial
Half Life
Recovery
Therapeutic Equivalence

DEPARTMENT: CLINICAL PHARMACOLOGY

DIVISION: DEVELOPMENTAL THERAPEUTICS

COMPARISON OF SAFETY AND HALF-LIFE OF SPECIALLY-PROCESSED FACTOR VIII WITH UNTREATED FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

PROTOCOL BB 1826-01

FINAL RESEARCH REPORT

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DATE April 15, 1983

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DATE April 18, 1983

COMPARISON OF SAFETY AND HALF-LIFE OF SPECIALLY-PROCESSED FACTOR VIII WITH UNTREATED FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

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COMPARISON OF SAFETY AND HALF-LIFE OF SPECIALLY-PROCESSED FACTOR VIII WITH UNTREATED FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

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RESEARCH AND DEVELOPMENT DIVISION

I. INTRODUCTION

The Revlon Health Care Group has treated Factor VIII derived from human plasma to reduce non-A, non-B Hepatitis activity and reduce the risk of Hepatitis B infection. All lots of Specially-Processed Factor VIII have been found to be negative for Hepatitis B by radioimmunoassay. This product should be of equal potency to untreated Factor VIII and have the potential of increased hepatitis safety. The present study was designed to evaluate the safety and efficacy of this product in hemophiliac patients with Hemophilia A.

II. TITLE

Comparison of Safety and Half-Life of Specially-Processed Factor VIII With Untreated Factor VIII in Patients with Hemophilia A.

III. INVESTIGATOR

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IV. OBJECTIVE

The objective of this study was to compare the safety, half-life and efficacy of Specially-Processed Factor VIII with untreated Factor VIII concentrate in patients with moderate or severe Hemophilia A (Factor VIII deficiency).

V. STUDY DESIGN

This open, balanced, 2-way crossover study required 6 home care patients in one center. The time per treatment was 48-72 hours per patient. Patients admitted into the study were home - care Hemophilia A patients who received a single infusion each of: 1) Specially-Processed Factor VIII, and 2) Untreated Factor VIII, separated by a washout period of 48-72 hours, which allowed their circulating Factor VIII: C levels to be less than or equal to 5% of the normal level. Both treatment medications (treated and untreated) were derived from the same lot of starting material.

VI. MATERIALS

Revlon Health Care Group supplied the Investigator with sufficient supplies of the following to allow for completion of this study.

*Specially-Processed Factorate, Generation I, lyophilized (Study Drug 1)	Lot # X 22602
*FACTORATE, Generation I, Untreated (Study Drug 2)	Lot # X 22502
Sterile Water for Injection, U.S.P., 10 ml.	Lot # X 31101

VII. PATIENT SELECTION

A total of 6 home care patients with documented Hemophilia A (Factor VIII deficiency) were required for completion of this study. The Principal Investigator recruited patients in numbers sufficient to

provide the requisite 6 evaluable, completed cases, employing the entry criteria detailed below.

Inclusion Criteria

A. Age: 12 years or older

B. Sex: Male

C. Weight: 25 kg (55 lb) or greater

D. Health Status: Patients had documented moderate or severe Hemophilia A (Factor VIII deficiency). Moderate deficiency was demonstrated by a baseline Factor VIII level of 1-5% activity (or 2-5 U/dl). Severe deficiency was documented by 0% Factor VIII activity (0-2 U/dl). Patients with circulating Factor VIII inhibitors were not entered into the study. Clinical and laboratory documentation in support of the patient's diagnosis was recorded in the patient's Case Report Form.

1. Patient Evaluation

At the time of screening, the Investigator obtained a complete medical history and performed a thorough physical examination including determination of vital signs and body weight.

2. Laboratory Tests

The following laboratory tests were obtained as a patient entered the study prior to infusion of Factor VIII.

Hemogram

Hemoglobin
Hematocrit

WBC (Total and Differential)
RBC
Platelet Count

Blood Chemistry

BUN
Creatinine
SGOT
Alkaline Phosphatase
LDH
Bilirubin (Total)
Calcium
Inorganic Phosphorus
Cholesterol
Total Protein
Albumin
Uric Acid
Glucose
Sodium
Potassium
Chloride
Bicarbonate
(HBSAg* and HBSAb* (IgG)) Hepatitis B Surface Antigen and Antibody

Urinalysis

Specific Gravity
pH
Ketones
Sugar
Protein
Microscopic Examination of Sediment

* Screening only

Coagulation, General

Prothrombin Time (PT)
Factor VIII Related Antigen, using Agarose Gel and Specific Antibody*
Factor VIII Ristocetin cofactor activity, using Aggregation of Formalin-Fixed Platelets*
Presence of Factor VIII inhibitors

Coagulation, Specific

Partial Thromboplastin Time (APTT)**
Factor VIII Procoagulant Activity**

Blood Bank

Direct Antiglobulin (Direct Coombs) Test

3. Electrocardiogram (ECG)

A standard 12-lead ECG was obtained from each patient. A copy of the electrocardiogram including a signed evaluation of the ECG was included in the Case Report Form. Only those patients with a normal ECG as judged by the Principal Investigator were accepted for entrance into this study.

**Obtained immediately prior to infusion of Specially-Processed Factor VIII.

* These studies may have been obtained prior to the patient's entry into the study, as part of a diagnostic workup.

NOTE: One-stage clotting times were used for all APTT and Factor VIII assays.

VIII. INFORMED CONSENT

Written informed consent forms were signed by all participating patients.

IX. PATIENT EXCLUSION

1. History of alcohol or drug abuse;

2. History of seizure disorder;

3. Thrombocytopenia;

4. History of chronic renal, hepatic, gastrointestinal, pulmonary or cardiovascular disease, or other chronic illness, exclusive of hepatitis;

5. The use of aspirin and aspirin-containing compounds within the past 10 days;

6. The presence of a bleeding disorder other than Hemophilia A;

7. Presence of a circulating Factor VIII inhibitor;

8. Participation in a drug research study within the past one month.

X. CLINICAL EVALUATION

1. History and Physical Examination

A medical history, including a history of drug abuse and weight determination, was obtained from each patient and recorded in the Case Report Form. A complete physical examination including

weight determination was conducted again prior to the second infusion and at the conclusion of the study.

2. Subjective Evaluation During Infusions

Patients were asked to report how they felt during and after each infusion. All medically significant comments were recorded in the Case Report Forms.

3. Vital Signs During Infusion

Blood pressure, temperature, pulse, and respiration were monitored during and after all infusions.

4. Infusion Site Evaluation

The infusion site was evaluated for discomfort, erythema, warmth, induration, and tenderness during and after each infusion.

XI. PROCEDURE

Patients were given a study number in consecutive order as they entered the study and were given infusions in the order set forth in Random Allocation Schedule. The elapsed time between the first and second infusions was 48 hours, or a time sufficient for the patient's circulating Factor VIII: C level to reach 5% of normal or below. Prior to infusion, all reconstituted vials were assayed in the Clinic Laboratory for Factor VIII potency, and the results were recorded in the patient's Case Report Forms.

A. INFUSIONS

Home - care patients with moderate to severe Hemophilia A who entered the study were given a single infusion of each study

medication under the direction of the Principal Investigator or Study Coordinator. Each patient received his accustomed prophylactic dose of either product. The number of units administered and the expected elevation in Factor VIII: C activity were recorded in the patient's Case Report Form. The observation period and biological sample collection for each of the two infusions continued over the 48 hours following the start of the infusion. Patients received their accustomed dose of each of the Factor VIII products, and the amount infused was recorded in the Case Report Form. The infusion site was evaluated at times 15 minutes and 1, 2 and 4 hours. Blood was taken for laboratory studies while patients remained in the Clinic at times zero, 15, 30 and 60 minutes and 2, 4, 6, and 8 hours. Patients were discharged at time 8 hours, and returned to the Clinic at times 24 and 48 hours post-infusion for the remaining determinations.

B. BIOLOGICAL SAMPLE COLLECTION

Wherever possible, blood samples were drawn from the contralateral arm.

1. Blood Chemistry, Hematology, Direct Antiglobulin Test and Urinalysis.

   These parameters were repeated at time 48 hours (prior to the second infusion) and at the conclusion of the study.

2. Coagulation studies.
   General (screening) coagulation studies were not repeated.

Partial Thromboplastin Time (APTT) and Factor VIII Procoagulant activity levels were drawn at or prior to time zero and were repeated at post-treatment times, 15, 30 and 60 minutes, and 2, 4, 6, 8, 24, and 48 hours.

C. PHYSICAL EXAMINATION

In addition to the screening physical examination, additional examination of each patient was conducted and recorded in the Case Report Form at appropriate times during the study. Physical examination included vital signs (oral temperature, pulse, respirations), and was performed prior to the second infusion and at the close of the study (48 hours post second infusion).

General Clinical Assessment

General Clinical Assessment including vital signs (blood pressure, temperature, pulse and respirations) was recorded at time: pre-infusion, 15 minutes, and 1, 2, and 4 hours, in the patient's Case Report Form. Vital signs were recorded with the patient sitting or supine in a consistant fashion.

D. INFUSION SITE EVALUATION

The infusion site was evaluated at time zero (pre-infusion) and post-infusion at times 15 minutes, 1, 2, and 4 hours.

The following parameters were evaluated:

1. Discomfort
2. Erythema
3. Warmth
4. Induration
5. Tenderness on palpation

E. SIDE EFFECTS

All side effects and adverse reactions were recorded on the Case Report Form according to date, time of occurrence, type, severity (mild, moderate, and severe) and duration. To avoid bias of suggestion, patients were asked, "how are you feeling?" The Investigator judged the severity of any side effects and recorded his evaluation on the Case Report Form.

F. DATA REPORTING

All data were recorded on individual-patient detailed Case Report Forms provided by Revlon Health Care Group.

G. STUDY TERMINATION

On the final day (48 hours after initiation of the last infusion), each patient received a complete physical examination and the battery of laboratory tests listed in Section VII, Patient Selection (with the exception of screening tests).

XII DATA EVALUATION

The estimate of the *in vivo* half-life for each patient was calculated as follows:

(a) using linear least square, a linear equation was estimated from the terminal phase of the Factor VIII activity level vs. time curve following the last dose.

(b) in vivo half-life ($t_{1/2}$) was estimated from the relation:

$$t_{1/2} = \frac{.693}{\beta}$$, where β is the slope of the Factor VIII activity vs. time profile.

(c) The Factor VIII: C levels were compared with the APTT values, and a correlation made.

(d) Recovery of Factor VIII was calculated from the equation:

$$K=\text{Recovery (u/dl rise/i.u./kg)}=\frac{\text{kg wt. x VIII rise (u/dl)}}{\text{dose (i.u.)}}$$

XIII. RESULTS AND DISCUSSION

1. Demographic Data

These data are presented on Table 1. Six adult males with moderate or severe Hemophilia A (less than 3% Factor VIII coagulant activity), ranging in ages from 22 to 50 years; entered and completed the study. No patient had a circulating inhibitor to Factor VIII. (See also Tables 2-7) Patient #5, aged 50, is mildly hypertensive, and is treated with hydrochlorthiazide, 50 mg q.d.

2. Electrocardiogram

All six patients had normal ECG's at screening. (See Table 8)

3. Screening Laboratory Data

Tables 2-7 present patients' coagulation history. All patients have severe or moderate Hemophilia A, with less than 3% Factor VIII activity, prolonged partial thromboplastin times and normal prothrombin times. They required a prophylactic dose of from 1020

to 1575 Units of Factor VIII in order to maintain hemostasis. All patients had antibody to Hepatitis B, indicating prior infection, but were Hepatitis B Surface Antigen negative. (See Table 8)

4. Blood Pressure

These values are presented in Tables 34-39. No significant changes in blood pressure occurred during or after infusions of either product. Note that patient #5 entered the study with mild hypertension, which did not change during treatment. (Table 38)

5. Heart Rate

See Tables 34-39. No significant changes in heart rate occurred during or after infusions of either product.

6. Respiration

No significant changes in respiration (Tables 34-39) were observed during infusions.

7. Temperature

No significant changes in temperature (Tables 34-39) were observed during or after infusions.

8. Infusion Site Evaluation

There were no local reactions at the infusion site as a result of infusions with either product. (Tables 40-45)

9. Hematology

(See Tables 21-26). There were no clinically significant

treatment-related perturbations of the hematological parameters measured. Patient 1's differential white blood cell count (See Table 21) shows elevated band cell and monocyte populations, which along with elevated liver function tests, may reflect non-A non-B hepatitis which predates the study. (See also Table 9). Patients 2, 3, 4, 5 and 6 also have minimal changes in differential white blood cell counts which are not felt to be clinically significant or drug related. (See Tables 22, 23, 24, 25 and 26). These changes consist of elevated relative monocyte and lymphocyte counts and relatively lowered neutrophil counts.

10. Blood Chemistry

(See Tables 9-14). Patient 1 shows elevated SGOT, SGPT, γ Glutamyl Transferase on screen and during the study, compatible with previous and current non-A, non-B hepatitis, according to the Principal Investigator. (See Table 9). Patients 2, 4, 5 and 6 show mildly elevated liver function tests (Tables 10, and 12 through 14), compatible with mild hepatic disease commonly seen in hemophilic patients. These elevations precede treatment.

Patients 1, 2, 5, and 6 (Tables 9, 10, 13, and 14) show mildly elevated total protein levels, which may reflect elevated globulin levels secondary to mild chronic hepatic inflammation. Patient 5, taking Hydrochlorthiazide, shows elevated glucose and uric acid and decreased potassium levels as a consequence of this therapy (see Table 13). Patient 6 also has elevated uric acid (Table 14), for unexplained reasons, not felt to be drug-related.

11. Coagulation

Each infusion lasted an average of 10 minutes. As a quality check on the potency of the Factor VIII vials, all vials were assayed for

Factor VIII content prior to or following infusion. The assayed potency of all vials used in this study is recorded by patient and by infusion on Tables 28 through 33. Figures 1 through 7 and Table 27 contain the plasma decay curves of Factor VIII plotted on a semilogarithmic scale and the calculated half-life and recovery values for all infusions. It can be seen from Figures 1 through 7 that the in vivo behavior of both the Specially-Processed and untreated Factor VIII are practically identical. The faster first phase of the curve (prior to 2 to 4 hours) represents the initial half disappearence time or redistribution phase, while the slower second phase, from 2-4 hours to 48 hours, is considered to represent the (degradative) biological half-life of Factor VIII. Table 27 shows the calculated half-life values for each infusion.

The second phase half-life means are 10.88 ± 4.11 hours for Specially-Processed Factor VIII, and 10.88 ± 3.57 hours for untreated Factor VIII, respectively. Recovery of both products is 1.8 ± 0.2 for Specially-Processed Factor VIII and 1.9 ± 0.2 for untreated Factor VIII , respectively. See Table 27 for the equation used to calculate recovery, and for recovery values.

Tables 28-33 show the partial thromboplastin time values for each infusion. It can be seen that the APTT approaches normal after infusions and gradually lengthens to near pre-infusion levels by 48 hours post-infusion.

12. Urinalysis

(See Tables 15-20). There are no clinically significant alterations in these parameters resulting from this study. The urine of patients 5 and 6 (Tables 19 and 20) contains uric acid crystals, in keeping with elevated serum uric acid levels (see Section XIII 10).

XIV. ADVERSE REACTIONS AND SIDE EFFECTS

None were observed during this study.

XV. CONCLUSIONS

Four severe and two moderate hemophilic patients were treated in a 2-way crossover design with untreated Factor VIII and specially-processed Factor VIII.

Half-life and recovery for both products are equivalent.

No adverse reactions or local intolerance to infusions were encountered.

No clinically significant perturbations of laboratory parameters resulted from treatment. Patients entering the study with elevated liver function tests probably had pre-existing mild hepatic dysfunction as is commonly encountered in the hemophilic population. Such findings are not drug-related.

(16) FACTORATE-01

BB 1826 - 01

RESEARCH AND DEVELOPMENT DIVISION

RANDOMIZATION SCHEDULE

SUBJECT*	LEG A	LEG B
1	2	1
2	2	1
3	1	2
4	1	2
5	2	1
6	1	2

TREATMENT

1 = Specially Processed Factor VIII

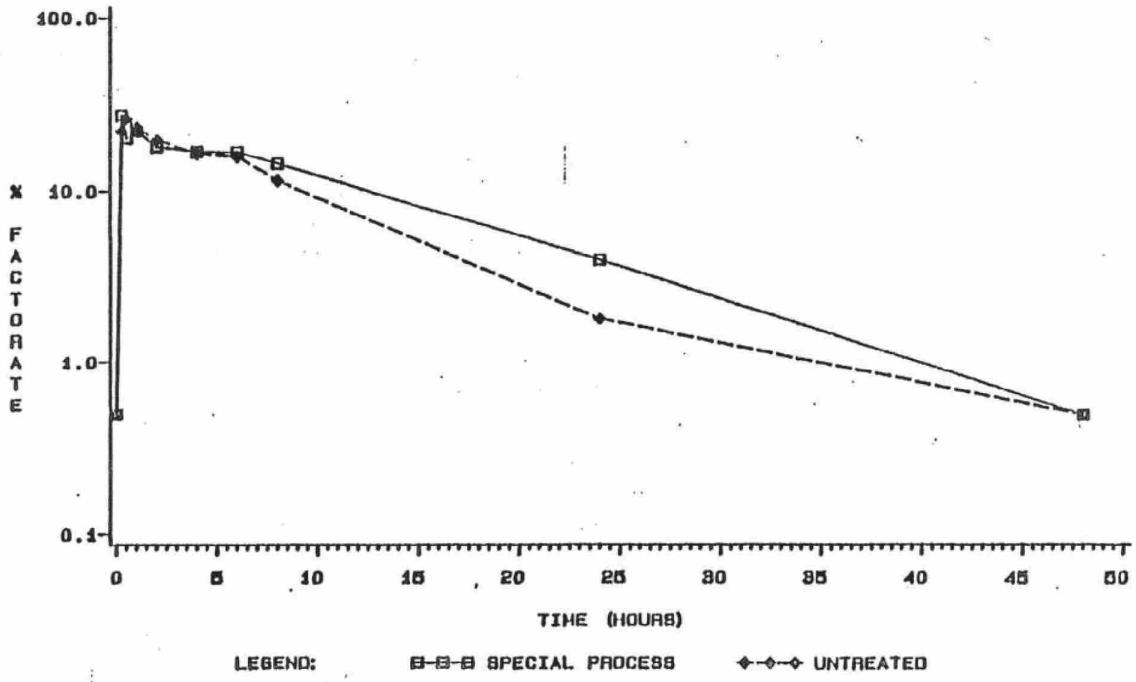
2 = Untreated Factor VIII

AP000513

FIGURE 1: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #1



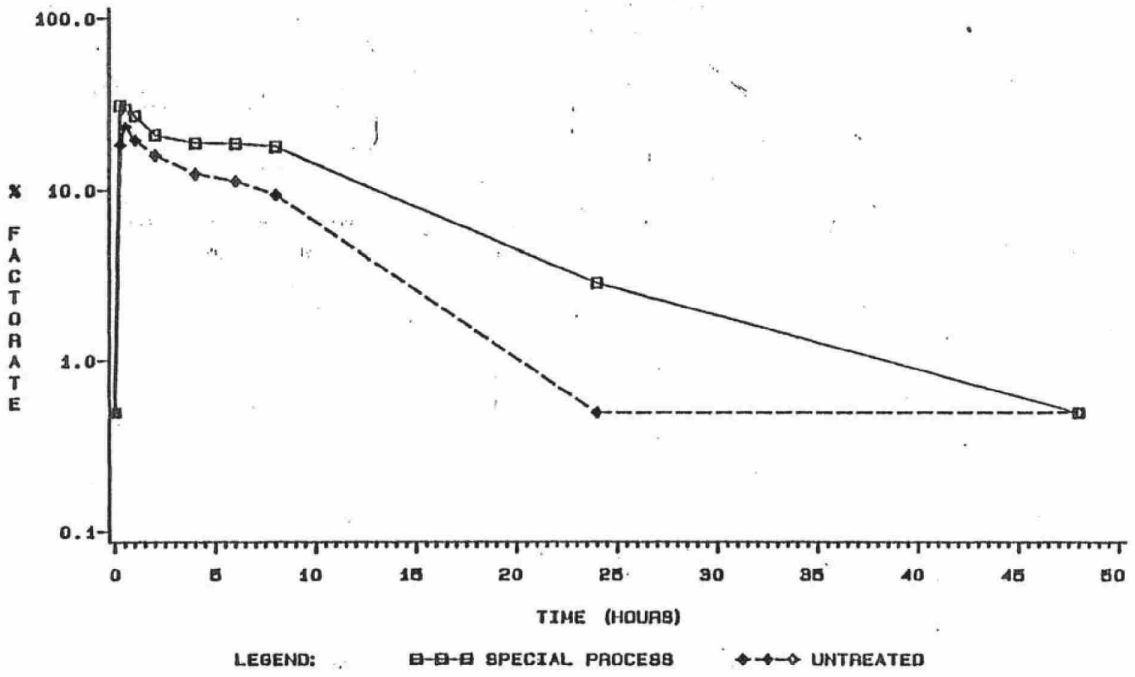
AP000514

ARMOUR001510

FIGURE 2: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #2



AP000515

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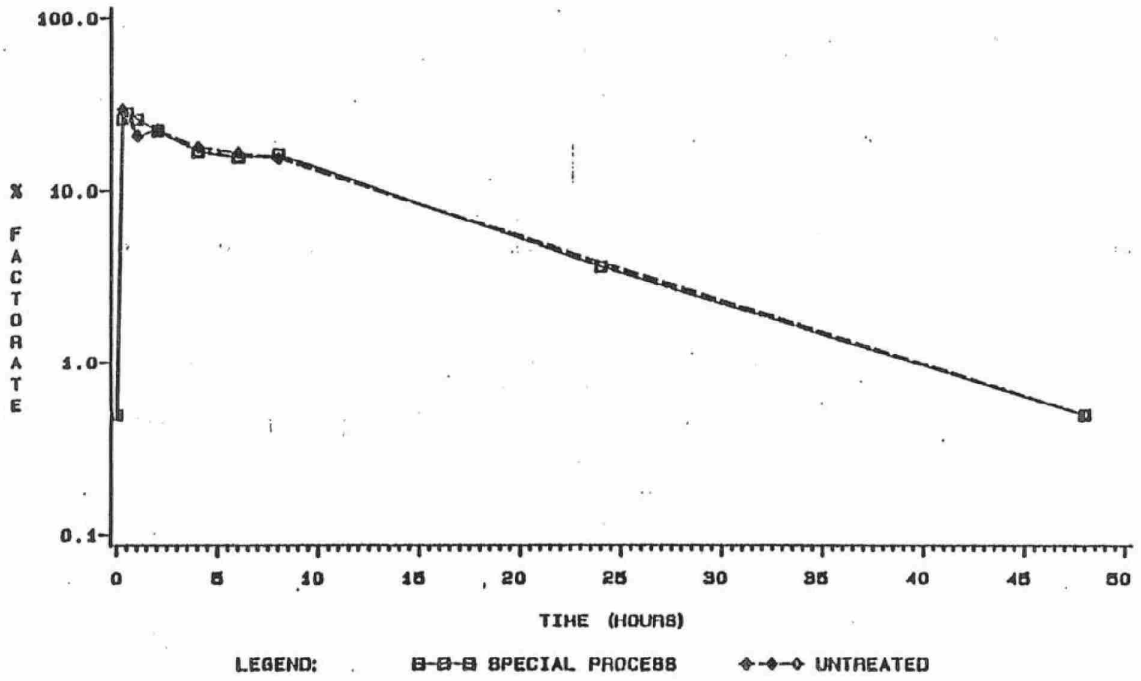
FIGURE 3: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #3



AP000516

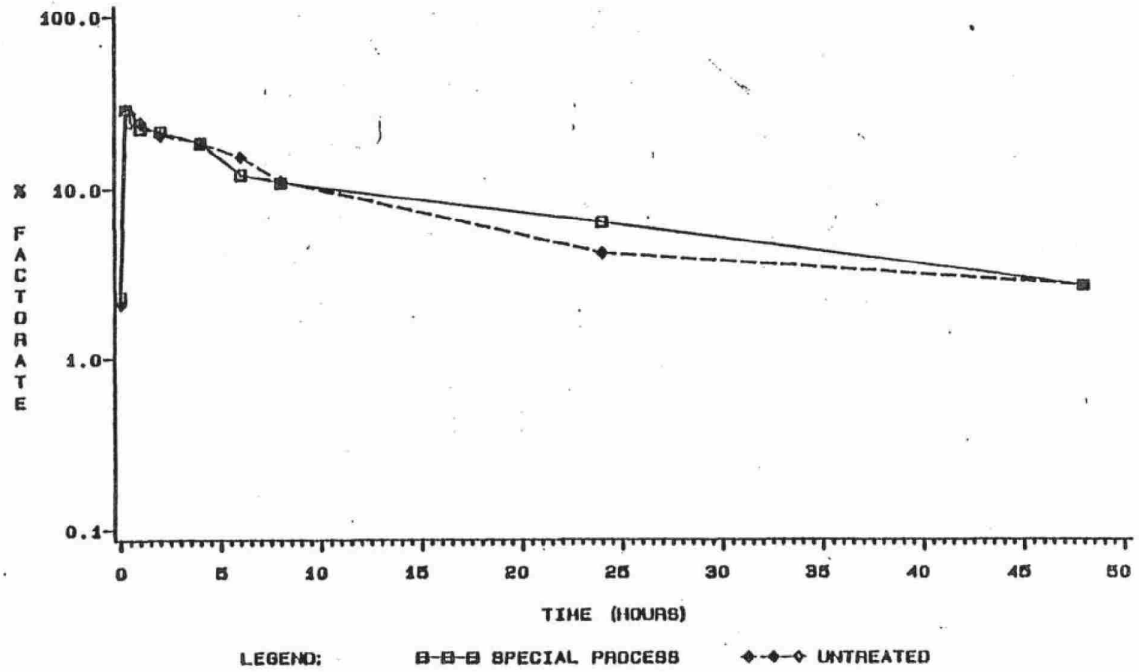
ARMOUR001512

ARMO0000164_0116

FIGURE 4: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #4



AP000517

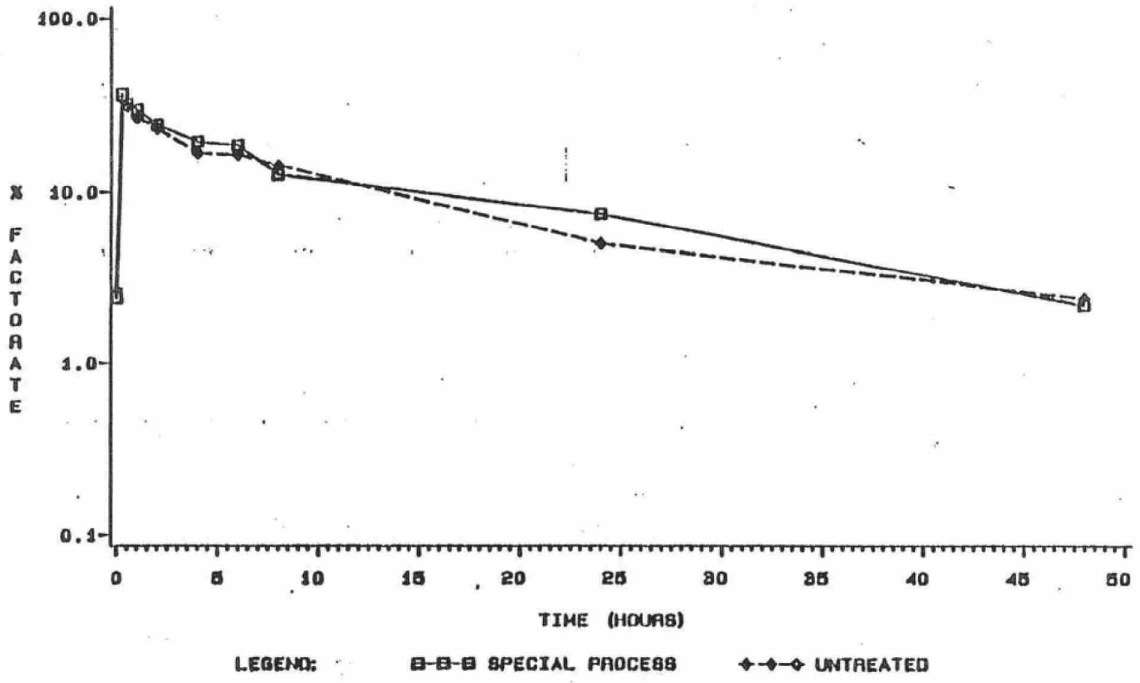
ARMOUR001513

ARMO0000164_0117

FIGURE 5: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #5



AP000518

ARMOUR001514

ARMO0000164_0118

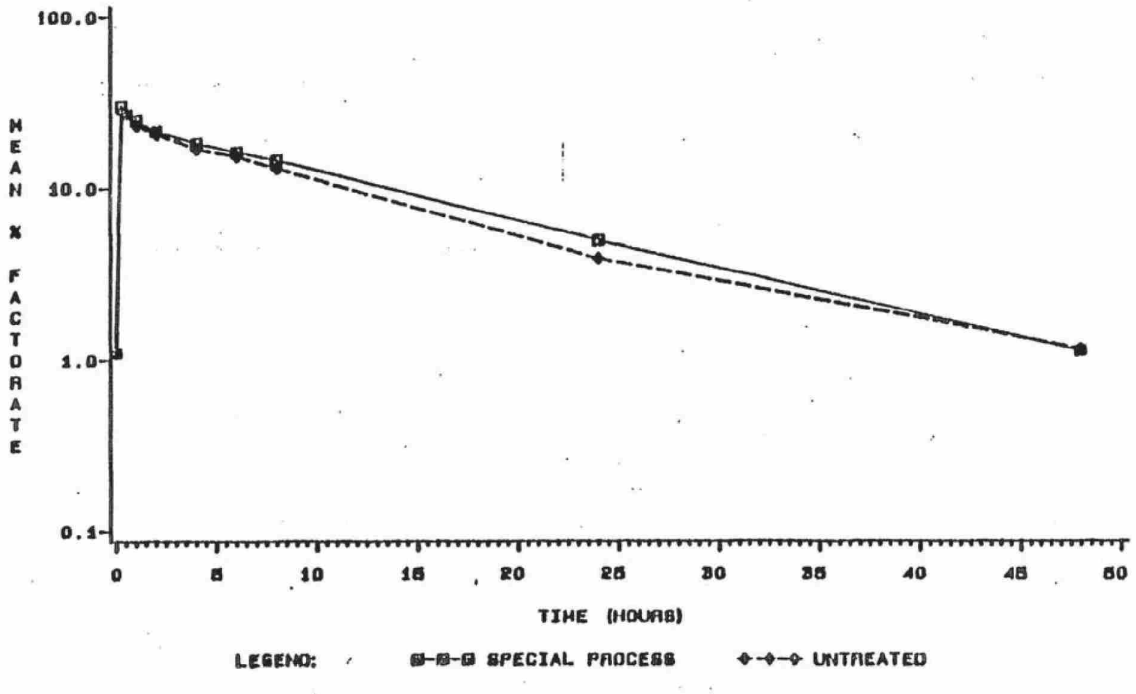
FIGURE 6: COMPARISON OF PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A

FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE

SUBJECT #6

AP000519

FIGURE 7: COMPARISON OF MEAN PLASMA LEVELS OF FACTOR VIII IN PATIENTS WITH HEMOPHILIA A
FOLLOWING INFUSION WITH SPECIALLY PROCESSED VS. UNTREATED FACTORATE
SUBJECTS #1-6



AP000520

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ARMOUR001516

ARMO0000164_0120

TABLE 1
FACTORATE - 01
BB 1826-01

DEMOGRAPHIC DATA

PATIENT	AGE	HEIGHT	WEIGHT	FRAME	FACTOR VIII LEVEL
1	24	173 cm	68 kg	Medium	Less than 1%
2	23	173 cm	68 kg	Medium	Less than 1%
3	22	177 cm	105.4 kg	Large	Less than 1%
4	27	175 cm	68 kg	Medium	2.3%
5	50	182 cm	88 kg	Medium	2.7%
6	28	185 cm	84 kg	Medium	Less than 1%

TABLE 2
FACTORATE - 01
BB 1826 - 01

PATIENT HISTORY

PATIENT 1

PROTHROMBIN TIME	11.3	SECONDS/CONTROL	11.2 SEC
PARTIAL THROMBOPLASTIN TIME	71.3	SECONDS/CONTROL	32.7 SEC
FACTOR VIII PROCOAGULANT ACTIVITY	<1	%	NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	199	U/dl	NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	127.8	U/dl	NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1020	UNITS	
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 7	DAYS	
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%	
PRODUCT LAST USED	ALPHA		

AP000522

TABLE 3
FACTORATE - 01
BB 1826 - 01

PATIENT HISTORY

PATIENT 2

PROTHROMBIN TIME	10.9	SECONDS/CONTROL 11.2 SEC	
PARTIAL THROMBOPLASTIN TIME	62.1	SECONDS/CONTROL 32.7 SEC	
FACTOR VIII PROCOAGULANT ACTIVITY	<1	%	NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	117.6	U/dl	NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	116.5	U/dl	NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1020	UNITS	
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 14	DAYS	
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%	
PRODUCT LAST USED	ALPHA		

AP000523

TABLE 4
FACTORATE - 01
BB 1826 - 01

PATIENT HISTORY

PATIENT 3

PROTHROMBIN TIME	10.4	SECONDS/CONTROL 11.2 SEC	
PARTIAL THROMBOPLASTIN TIME	78.3	SECONDS/CONTROL 32.7 SEC	
FACTOR VIII PROCOAGULANT ACTIVITY	<1	%	NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	216	U/dl	NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	77.8	U/dl	NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1575	UNITS	
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 5	DAYS	
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%	
PRODUCT LAST USED	ARMOUR		

AP000524

ARMOUR001520

ARMO0000164_0124

TABLE 5
FACTORATE - 01
BB 1826 - 01

PATIENT HISTORY

PATIENT 4

PROTHROMBIN TIME	11.4	SECONDS/CONTROL 11.6 SEC	
PARTIAL THROMBOPLASTIN TIME	64.3	SECONDS/CONTROL 32 SEC	
FACTOR VIII PROCOAGULANT ACTIVITY	2.3	%	NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	116.7	U/dl	NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	116.5	U/dl	NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1020	UNITS	
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 30	DAYS	
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%	
PRODUCT LAST USED	CUTTER		

AP000525

ARMOUR001521

TABLE 6
FACTORATE - 01
BB 1826 - 01

PATIENT HISTORY

PATIENT 5

PROTHROMBIN TIME	11.0	SECONDS/CONTROL 11.6 SEC
PARTIAL THROMBOPLASTIN TIME	68.0	SECONDS/CONTROL 32.0 SEC
FACTOR VIII PROCOAGULANT ACTIVITY	2.7	% NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	162	U/dl NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	158	U/dl NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1320	UNITS
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 5	DAYS
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%
PRODUCT LAST USED	ARMOUR	

TABLE 7
FACTORATE - 01
BB 1876 - 01

PATIENT HISTORY

PATIENT 6

PROTHROMBIN TIME	10.6	SECONDS/CONTROL	11.6 SEC
PARTIAL THROMBOPLASTIN TIME	70.5	SECONDS/CONTROL	32.0 SEC
FACTOR VIII PROCOAGULANT ACTIVITY	<1	%	NORMAL 50-200%
FACTOR VIII RELATED ANTIGEN	170	U/dl	NORMAL 50-200 U/dl
FACTOR VIII RISTOCETIN COFACTOR ACTIVITY	137	U/dl	NORMAL 50-200 U/dl
CUSTOMARY PROPHYLACTIC FACTOR VIII DOSE	1260	UNITS	
CUSTOMARY FREQUENCY OF PROPHYLAXIS	Q 7	DAYS	
EXPECTED RISE IN FACTOR VIII ACTIVITY	30	%	
PRODUCT LAST USED	CUTTER		

AP000527

TABLE 8

FACTORATE - 01

BB 1826-01

SCREENING DATA

PATIENT	HBSAg	HBSAb	ECG
1	neg	pos	normal
2	neg	pos	normal
3	neg	pos	normal
4	neg	pos	normal
5	neg	pos	normal
6	neg	pos	normal

TABLE 9

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 1

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	139	135	142	135-145 mEq/L
POTASSIUM	3.6	4.2	4.3	3.5-5.0 mEq/L
CHLORIDE	102	104	108	95-105 mEq/L
BICARBONATE	30.0	21.0	23.0	22.0-30.0 mEq/L
CALCIUM	9.5	9.5	10.2	8.5-10.5 mg/dl
PHOSPHOROUS	2.3	3.0	2.8	2.5-4.5 mg/dl
BUN	14.0	12.0	11.0	10.0-26.0 mg/dl
URIC ACID	6.4	4.7	4.9	3.4-7.0 mg/dl
GLUCOSE	77	76	90	70-105 mg/dl
TOTAL PROTEIN	8.10	8.40	8.70	6.00-8.00 g/dl
ALBUMIN	4.40	4.60	4.50	3.30-5.20 g/dl
TOTAL BILIRUBIN	0.8	0.7	1.0	0.2-1.0 mg/dl
SGOT	450	610	400	10.0-40.0 IU/L
ALK PHOS	110	116	110	30-115 IU/L
LDH	185	195	210	100-225 IU/L
CHOLESTEROL	167	160	162	120-240 mg/dl
CREATININE	0.9	0.8	0.9	0.7-1.4 mg/dl
SGPT	975	1190	1125	0-48 IU/L
GLUTAMYL TRANSFERASE	103	115	125	11-51 IU/L

TABLE 10

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 2

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	140	141	143	135-145 mEq/L
POTASSIUM	4.2	4.2	4.1	3.5-5.0 mEq/L
CHLORIDE	106	107	107	95-105 mEq/L
BICARBONATE	26.0	24.0	27.0	22.0-30.0 mEq/L
CALCIUM	9.7	9.7	9.6	8.5-10.5 mg/dl
PHOSPHOROUS	2.6	3.8	3.1	2.5-4.5 mg/dl
BUN	13	14	16	10.0-26.0 mg/dl
URIC ACID	5.7	5.5	5.6	3.4-7.0 mg/dl
GLUCOSE	93	78	92	70-105 mg/dl
TOTAL PROTEIN	7.7	8.2	8.0	6.00-8.00 g/dl
ALBUMIN	4.4	4.6	4.5	3.30-5.20 g/dl
TOTAL BILIRUBIN	0.7	0.7	0.9	0.2-1.0 mg/dl
SGOT	55	65	62	10.0-40.0 IU/L
ALK PHOS	83	100	85	30-115 IU/L
LDH	150	150	140	100-225 IU/L
CHOLESTEROL	160	155	146	120-240 mg/dl
CREATININE	0.9	0.8	1.0	0.7-1.4 mg/dl
SGPT	95	115	118	0-48 IU/L
γGLUTAMYL TRANSFERASE	20	20	23	11-51 IU/L

TABLE 11

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 3

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	141	139	138	135-145 mEq/L
POTASSIUM	3.8	3.8	4.1	3.5-5.0 mEq/L
CHLORIDE	109	108	107	95-105 mEq/L
BICARBONATE	24.0	24.0	23.0	22.0-30.0 mEq/L
CALCIUM	9.4	9.5	9.8	8.5-10.5 mg/dl
PHOSPHOROUS	2.6	3.2	2.7	2.5-4.5 mg/dl
BUN	9.0	10.0	12.0	10.0-26.0 mg/dl
URIC ACID	6.0	5.2	5.9	3.4-7.0 mg/dl
GLUCOSE	105	92	100	70-105 mg/dl
TOTAL PROTEIN	7.4	7.7	7.9	6.00-8.00 g/dl
ALBUMIN	4.3	4.5	4.6	3.30-5.20 g/dl
TOTAL BILIRUBIN	0.7	0.5	0.8	0.2-1.0 mg/dl
SGOT	30	30	37	10.0-40.0 IU/L
ALK PHOS	60	50	52	30-115 IU/L
LDH	225	215	190	100-225 IU/L
CHOLESTEROL	130	140	166	120-240 mg/dl
CREATININE	0.9	0.8	1.0	0.7-1.4 mg/dl
SGPT	45	40	40	0-48 IU/L
γGLUTAMYL TRANSFERASE	15	17	17	11-51 IU/L

TABLE 12

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 4

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	141	144	143	135-145 mEq/L
POTASSIUM	3.7	3.9	4.1	3.5-5.0 mEq/L
CHLORIDE	105	107	105	95-105 mEq/L
BICARBONATE	26.0	28.0	27.0	22.0-30.0 mEq/L
CALCIUM	9.4	9.4	10.2	8.5-10.5 mg/dl
PHOSPHOROUS	2.4	2.8	3.1	2.5-4.5 mg/dl
BUN	12	9	14	10.0-26.0 mg/dl
URIC ACID	4.9	5.2	5.6	3.4-7.0 mg/dl
GLUCOSE	95	76	72	70-105 mg/dl
TOTAL PROTEIN	7.6	7.3	8.0	6.00-8.00 g/dl
ALBUMIN	4.3	4.4	4.4	3.30-5.20 g/dl
TOTAL BILIRUBIN	0.6	0.6	0.6	0.2-1.0 mg/dl
SGOT	55	45	55	10.0-40.0 IU/L
ALK PHOS	47	50	55	30-115 IU/L
LDH	175	185	215	100-225 IU/L
CHOLESTEROL	155	140	165	120-240 mg/dl
CREATININE	1.0	0.9	0.8	0.7-1.4 mg/dl
SGPT	85	80	95	0-48 IU/L
γGLUTAMYL TRANSFERASE	33	31	35	11-51 IU/L

TABLE 13

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 5

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	140	139	143	135-145 mEq/L
POTASSIUM	2.7	2.7	2.8	3.5-5.0 mEq/L
CHLORIDE	100	96	98	95-105 mEq/L
BICARBONATE	28.0	31.0	31.0	22.0-30.0 mEq/L
CALCIUM	9.90	10.20	10.20	8.5-10.5 mg/dl
PHOSPHOROUS	2.70	3.00	3.30	2.5-4.5 mg/dl
BUN	13	16	16	10.02-6.0 mg/dl
URIC ACID	8.90	8.00	7.00	3.4-7.0 mg/dl
GLUCOSE	113	99	107	70-105 mg/dl
TOTAL PROTEIN	8.3	9.0	8.6	6.00-8.00 g/dl
ALBUMIN	4.6	5.0	4.6	3.30-5.20 g/dl
TOTAL BILIRUBIN	1.3	1.8	1.5	0.2-1.0 mg/dl
SGOT	76	80	80	10.0-40.0 IU/L
ALK PHOS	64	62	65	30-115 IU/L
LDH	177	185	200	100-225 IU/L
CHOLESTEROL	220	240	200	120-240 mg/dl
CREATININE	1.1	1.1	1.1	0.7-1.4 mg/dl
SGPT	164	130	130	0-48 IU/L
γGLUTAMYL TRANSFERASE	158	167	140	11-51 IU/L

TABLE 14

FACTORATE - 01
BB 1826 -01

BLOOD CHEMISTRY

PATIENT 6

TEST	SCREEN	48 HRS	DISCHARGE	UNITS NL RANGE
SODIUM	140	142	140	135-145 mEq/L
POTASSIUM	4.0	4.2	4.2	3.5-5.0 mEq/L
CHLORIDE	107	107	106	95-105 mEq/L
BICARBONATE	23	28	27	22.0-30.0 mEq/L
CALCIUM	9.3	9.9	9.9	8.5-10.5 mg/dl
PHOSPHOROUS	3.5	4.0	4.1	2.5-4.5 mg/dl
BUN	8	12	14	10.0-26.0 mg/dl
URIC ACID	6.7	9.2	8.7	3.4-7.0 mg/dl
GLUCOSE	91	66	82	70-105 mg/dl
TOTAL PROTEIN	7.8	9.5	8.0	6.00-8.00 g/dl
ALBUMIN	4.0	4.4	4.1	3.30-5.20 g/dl
TOTAL BILIRUBIN	0.4	0.4	0.6	0.2-1.0 mg/dl
SGOT	49	95	75	10.0-40.0 IU/L
ALK PHOS	90	85	75	30-115 IU/L
LDH	165	210	200	100-225 IU/L
CHOLESTEROL	184	190	170	120-240 mg/dl
CREATININE	0.8	0.9	0.8	0.7-1.4 mg/dl
SGPT	73	107	105	0-48 IU/L
γGLUTAMYL TRANSFERASE	40	51	50	11-51 IU/L

TABLE 15

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 1

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	6.0	7.0	8.0	--
SPECIFIC GRAVITY	1.020	1.020	1.015	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	0	0	0	000
WBC's	1	1	1	000
CASTS	0	0	0	000
CRYSTALS	0	0	1 per HPF Triple Phosphate	000
CELLS	1 per HPF epithelial	0	0	000

TABLE 16

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 2

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	8.0	6.0	7.0	--
SPECIFIC GRAVITY	1.020	1.026	1.020	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	1	0	0	0
WBC's	1	0	0	0
CASTS	0	0	0	0
CRYSTALS	0	0	Triple Phosphate	000
CELLS	Epithelial	0	0	0

TABLE 17

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 3

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	6.0	7.0	7.0	--
SPECIFIC GRAVITY	1.022	1.020	1.020	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	0	0	0	0
WBC's	1	0	0	0
CASTS	0	0	0	0
CRYSTALS	0	0	0	0
CELLS	Epithelial	0	0	0

TABLE 18

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 4

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	6.0	6.0	7.0	---
SPECIFIC GRAVITY	1.026	1.020	1.020	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	1	1	1	0
WBC's	1	1	0	0
CASTS	0	0	0	0
CRYSTALS	0	0	0	0
CELLS	0	0	Epithelial	0

TABLE 19

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 5

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	7.0	7.0	5.0	--
SPECIFIC GRAVITY	1.015	1.010	1.015	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	1	0	1	0
WBC's	0	0	0	0
CASTS	0	0	0	0
CRYSTALS	0	0	Urates	0
CELLS	0	0	0	0

TABLE 20

FACTORATE - 01
BB 1826 -01

URINALYSIS

PATIENT 6

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
pH	5.0	6.0	7.0	—
SPECIFIC GRAVITY	1.020	1.020	1.020	1.001-1.035
PROTEIN	0	0	0	0
SUGAR	0	0	0	0
RBC's	0	0	0	0
WBC's	0	0	0	0
CASTS	0	0	0	0
CRYSTALS	0	0	Urates	0
CELLS	0	0	0	0

TABLE 21

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 1

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	5.83	5.97	5.79	$4.7-6.1 \times 10^6/mm^3$
HEMOGLOBIN	17.7	18.1	17.4	14.0-18.0 g/dl
HEMATOCRIT	53.6	54.7	52.5	42 - 52%
MCV	92	92	90	80 - 94 u^3
MCH	30.4	30.3	30.1	27 -31 u ug
MCHC	33.0	33.1	33.2	32 -36%
PLATELETS	157,000	157,000	213,000	$150-500 \times 10^3/mm^3$
TOTAL WBC	6500	6900	7500	$4.8-10.8 \times 10^3/mm^3$
NEUTROPHILS	59	52	37	60 - 70%
BAND CELLS	0	0	14	0 - 10%
LYMPHOCYTES	28	36	29	25 - 33%
ATYPICAL LYMPHS	0	0	5	0 - 10%
MONOCYTES	13	11	15	2 - 6%
EOSINOPHILS	1	1	0	1 - 4%
BASOPHILS	0	0	0	0.0 - 0.5%
OTHER	—	—	—	—
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	—

TABLE 22

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 2

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	5.46	5.60	5.14	$4.7\text{-}6.1 \times 10^6/mm^3$
HEMOGLOBIN	16.8	16.8	16.2	14.0-18.0 g/dl
HEMATOCRIT	49.5	49.9	47.2	42 - 52%
MCV	91	89	91	80 - 94 u^3
MCH	30.8	30.0	31.6	27 -31 u ug
MCHC	33.9	33.7	34.4	32 -36%
PLATELETS	157,000	176,000	209,000	$150\text{-}500 \times 10^3/mm^3$
TOTAL WBC	5000	5700	5400	$4.8\text{-}10.8 \times 10^3/mm^3$
NEUTROPHILS	42	50	34	60 - 70%
BAND CELLS	0	1	6	0 - 10%
LYMPHOCYTES	42	33	40	25 - 33%
ATYPICAL LYMPHS	0	0	5	0 - 10%
MONOCYTES	11	12	12	2 - 6%
EOSINOPHILS	4	2	3	1 - 4%
BASOPHILS	1	2	0	0.0 - 0.5%
OTHER	—	—	—	—
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	—

TABLE 23

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 3

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	6.05	5.73	5.69	$4.7\text{-}6.1 \times 10^6/mm^3$
HEMOGLOBIN	16.1	15.7	15.7	14.0-18.0 g/dl
HEMATOCRIT	50.7	47.6	48.0	42 - 52%
MCV	84	83	85	80 - 94 u^3
MCH	26.6	27.4	27.9	27 -31 u ug
MCHC	31.8	33.0	32.8	32 -36%
PLATELETS	411,000	479,000	514,000	$150\text{-}500 \times 10^3/mm^3$
TOTAL WBC	12,000	10,500	11,400	$4.8\text{-}10.8 \times 10^3/mm^3$
NEUTROPHILS	46	51	43	60 - 70%
BAND CELLS	0	0	6	0 - 10%
LYMPHOCYTES	46	42	34	25 - 33%
ATYPICAL LYMPHS	0	0	4	0 - 10%
MONOCYTES	7	7	12	2 - 6%
EOSINOPHILS	1	0	1	1 - 4%
BASOPHILS	0	0	0	0.0 - 0.5%
OTHER	—	—	—	—
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	—

TABLE 24

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 4

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	5.41	5.36	5.30	$4.7-6.1 \times 10^6/mm^3$
HEMOGLOBIN	16.3	16.3	15.7	14.0-18.0 g/dl
HEMATOCRIT	47.8	47.7	48.2	42 - 52%
MCV	88	89	90	80 - 94 u^3
MCH	30.1	30.4	29.7	27 -31 u ug
MCHC	34.1	34.2	32.6	32 -36%
PLATELETS	210,000	244,000	254,000	$150-500 \times 10^3/mm^3$
TOTAL WBC	3900	5500	5000	$4.8-10.8 \times 10^3/mm^3$
NEUTROPHILS	54	57	46	60 - 70%
BAND CELLS	0	0	2	0 - 10%
LYMPHOCYTES	33	31	36	25 - 33%
ATYPICAL LYMPHS	2	0	0	0 - 10%
MONOCYTES	6	7	12	2 - 6%
EOSINOPHILS	5	5	4	1 - 4%
BASOPHILS	0	0	0	0.0 - 0.5%
OTHER	---	---	---	---
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	---

TABLE 25

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 5

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	5.82	5.86	5.47	4.7-6.1x10^6/mm^3
HEMOGLOBIN	18.1	17.9	16.5	14.0-18.0 g/dl
HEMATOCRIT	51.1	52.1	49.5	42 - 52%
MCV	88	89	90	80 - 94 u^3
MCH	31.1	30.5	30.1	27 -31 u ug
MCHC	35.4	34.4	33.2	32 -36%
PLATELETS	202,000	172,000	222,000	150-500x10^3/mm^3
TOTAL WBC	6800	6100	6200	4.8-10.8x10^3/mm^3
NEUTROPHILS	65	60	39	60 - 70%
BAND CELLS	2	1	1	0 - 10%
LYMPHOCYTES	21	31	36	25 - 33%
ATYPICAL LYMPHS	0	1	0	0 - 10%
MONOCYTES	12	5	16	2 - 6%
EOSINOPHILS	0	1	7	1 - 4%
BASOPHILS	0	2	1	0.0 - 0.5%
OTHER				—
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	—

TABLE 26

FACTORATE - 01
BB 1826 -01

HEMATOLOGY

PATIENT 6

TEST	SCREEN	48 HRS	DISCHARGE	NORMAL RANGE
TOTAL RBC	5.63	5.65	5.21	$4.7\text{-}6.1 \times 10^6/mm^3$
HEMOGLOBIN	15.0	15.9	14.0	14.0-18.0 g/dl
HEMATOCRIT	46.9	47.6	43.4	42 - 52%
MCV	83	84	83	80 - 94 u^3
MCH	26.6	28.1	26.8	27 -31 u ug
MCHC	32.0	33.4	32.2	32 -36%
PLATELETS	232,000	238,000	225,000	$150\text{-}500 \times 10^3/mm^3$
TOTAL WBC	6600	6100	5800	$4.8\text{-}10.8 \times 10^3/mm^3$
NEUTROPHILS	65	63	56	60 - 70%
BAND CELLS	0	0	6	0 - 10%
LYMPHOCYTES	24	30	27	25 - 33%
ATYPICAL LYMPHS	0	0	0	0 - 10%
MONOCYTES	7	7	7	2 - 6%
EOSINOPHILS	4	0	4	1 - 4%
BASOPHILS	0	0	0	0.0 - 0.5%
OTHER	0	0	0	---
RED CELL MORPHOLOGY	NORMAL	NORMAL	NORMAL	---

HALF-LIFE AND RECOVERY OF FACTOR VIII

PATIENT	TREATMENT	WEIGHT (kg)	DOSE (i.u.)	MAXIMUM	HALF - LIFE (HOURS)	RECOVERY*
1	SPECIALLY PROCESSED	68.0	1140	27.5	8.5	1.6
	UNTREATED	68.0	975	26.2	8.3	1.8
2	SPECIALLY PROCESSED	68.0	1140	31.0	8.0	1.8
	UNTREATED	68.0	975	23.2	9.4	1.6
3	SPECIALLY PROCESSED	105.4	1710	28.2	8.4	1.7
	UNTREATED	105.4	1625	29.7	8.3	1.9
4	SPECIALLY PROCESSED	68.0	1140	26.9	17.7	1.6
	UNTREATED	68.0	975	27.4	16.0	1.9
5	SPECIALLY PROCESSED	88.0	1425	34.8	14.2	2.1
	UNTREATED	88.0	1300	31.6	14.9	2.1
6	SPECIALLY PROCESSED	84.0	1425	31.5	8.5	1.9
	UNTREATED	84.0	1300	35.2	8.4	2.3

MEAN ± S.D.	SPECIALLY PROCESSED	10.88 ± 4.11	1.8 ± .2
	UNTREATED	10.88 ± 3.57	1.9 ± .2

*RECOVERY (K) IS CALCULATED AS FOLLOWS:

$$K(\text{u/dl rise/i.u./kg}) = \frac{\text{kg body weight x Factor VIII rise (u/dl)}}{\text{dose of Factor VIII administered (u)}}$$

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FACTORATE - 01
BB 1826 -01

COAGULATION STUDIES

PATIENT 1

INFUSION 1 DOSE: 975 Units			
PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 325 Units Assayed 331.5 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.
SCR	71.3/32.7	<1%	NEG
15 MIN	43.2/32.7	22.3%	
30 MIN	46.7/32.7	26.2%	
1 HR	46.2/32.7	23.4%	
2 HRS	46.2/32.7	20.0%	
4 HRS	47.5/32.7	16.5%	
6 HRS	48.0/32.7	15.9%	
8 HRS	49.6/32.7	11.6%	
24 HRS	57.1/32.3	1.8%	
48 HRS	64.2/32.3	<1.0%	NEG

INFUSION 2 DOSE: 1140 Units			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII			
POTENCY: Labeled 285 Units Assayed 262.2 Units			
TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	64.2/32.3	<1%	NEG
15 MIN	43.4/32.3	27.5%	
30 MIN	43.6/32.3	20.4%	
1 HR	43.3/32.3	22 4%	
2 HRS	43.7/32.3	18.0%	
4 HRS	45.9/32.3	17.0%	
6 HRS	47.5/32.3	16.9%	
8 HRS	46.1/32.3	14.5%	
24 HRS	52.9	4.0%	
48 HRS	62.0	<1%	NEG
DSC	-	<1%	NEG

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BB 1826 -01

COAGULATION STUDIES

PATIENT 2

INFUSION 1 DOSE: 975 Units			
PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 325 Units Assayed 305 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.
SCR	62.1/32.7	<1%	NEG
15 MIN	38.7/32.7	18.2%	
30 MIN	38.8/32.7	23.2%	
1 HR	39.4/32.7	19.7%	
2 HRS	40.0/32.7	16.1%	
4 HRS	43.7/32.7	12.6%	
6 HRS	43.8/32.7	11.5%	
8 HRS	44.2/32.7	9.5%	
24 HRS	52.3/32.3	< 1%	
48 HRS	59.5/32.3	<1%	NEG

INFUSION 2 DOSE: 1140 Units			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII			
POTENCY: Labeled 285 Units Assayed 265.6 Units			
TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	59.5/32.3	< 1%	NEG
15 MIN	37.5/32.3	31%	
30 MIN	37.7/32.3	29.5%	
1 HR	37.5/32.3	27.2%	
2 HRS	40.1/32.3	21.1%	
4 HRS	40.5/32.3	18.8%	
6 HRS	41.1/32.3	18.7%	
8 HRS	43.6/32.3	17.9%	
24 HRS	52.0	2.9%	
48 HRS	57.3	< 1%	
DSC	-	<1%	NEG

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COAGULATION STUDIES

PATIENT 3

INFUSION 1 DOSE: 1710 Units			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII			
POTENCY: Labeled 285 Units Assayed 296 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.
SCR	78.3/32.7	< 1%	NEG
15 MIN	35.4/32.7	26%	
30 MIN	35.6/32.7	28.2%	
1 HR	36.6/32.7	26%	
2 HRS	36.5/32.7	22.4%	
4 HRS	36.6/32.7	16.9%	
6 HRS	38.0/32.7	15.7%	
8 HRS	39.7/32.3	16.4%	
24 HRS	47.9/32.3	3.7%	
48 HRS	54.7/32.3	<1%	NEG

INFUSION 2 DOSE: 1625 Units			
PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 325 Units Assayed 293 Units			
TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	54.7/32.3	< 1%	NEG
15 MIN	36.8/32.3	19.7%	
30 MIN	37.2/32.3	26.6%	
1 HR	37.7/32.3	21.0%	
2 HRS	38.0/32.3	22.7%	
4 HRS	39.4/32.3	17.8%	
6 HRS	40.1/32.3	16.7%	
8 HRS	42.2/32.3	15.5%	
24 HRS	SPECIMEN LOST		
48 HRS	56.0	< 1%	NEG
DSC	-	<1%	NEG

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COAGULATION STUDIES

PATIENT 4

INFUSION 1 DOSE: 1140 UNITS				INFUSION 2 DOSE: 975 UNITS			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII				PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 325 Units Assayed 289 Units				POTENCY: Labeled 285 Units Assayed 261 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.	TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	64.3/32	2.3%	NEG	SCR	56.7/32.2	2.1%	NEG
15 MIN	41.8/32	29.2%		15 MIN	45.0/32.2	29.5%	
30 MIN	42.2/32	24.6%		30 MIN	43.3/32.2	28.1%	
1 HR	43.6/32	22.6%		1 HR	44.1/32.2	24.7%	
2 HRS	42.8/32	21.8%		2 HRS	46.3/32.2	20.7%	
4 HRS	45.7/32	18.5%		4 HRS	45.8/32.2	18.7%	
6 HRS	45.7/32	12.2%		6 HRS	47.3/32.2	15.5%	
8 HRS	46.9/32	11.0%		8 HRS	49.6/33.7	11.3%	
24 HRS	52.6/32	6.4%		24 HRS	46.1/32.2	4.2%	
48 HRS	56.7/33.7	2.7%	NEG	48 HRS	50.0/33.7	2.7%	NEG
				DSC	50/33.7	2.7%	NEG

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COAGULATION STUDIES

PATIENT 5

INFUSION 1 DOSE: 1300 UNITS			
PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 285 Units Assayed 276 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.
SCR	68.0/32.0	2.75	NEG
15 MIN	42.0/32.0	34.3%	
30 MIN	41.9/32.0	31.1%	
1 HR	42.7/32.0	27.2%	
2 HRS	42.9/32.0	23.5%	
4 HRS	43.1/32.0	16.9%	
6 HRS	45.3/32.0	16.6%	
8 HRS	45.4/32.0	14.4%	
24 HRS	53.0/32.0	5.1%	
48 HRS	59.9/33.7	2.4%	NEG

INFUSION 2 DOSE: 1425 UNITS			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII			
POTENCY: Labeled 325 Units Assayed 306 Units			
TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	59.9/32.2	2.4%	NEG
15 MIN	42.5/32.2	37.2%	
30 MIN	42.6/32.2	32.5%	
1 HR	42.2/32.2	30.1%	
2 HRS	42.3/32.2	24.6%	
4 HRS	43.2/32.2	19.8%	
6 HRS	44.4/32.2	18.9%	
8 HRS	45.7/32.2	12.7%	
24 HRS	51.0/33.7	7.5%	
48 HRS	60.0/33.7	2.2%	NEG
DSC	60.0/33.7	2.2%	NEG

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COAGULATION STUDIES

PATIENT 6

INFUSION 1 DOSE: 1425 UNITS				INFUSION 2 DOSE: 1300 UNITS			
PRODUCT: SPECIALLY-PROCESSED FACTOR VIII				PRODUCT: UNTREATED FACTORATE			
POTENCY: Labeled 285 Units Assayed 269 Units				POTENCY: Labeled 325 Units Assayed 310 Units			
TIME	APTT	FACTOR VIII LEVEL	D.A.T.	TIME	APTT SEC	FACTOR VIII LEVEL	D.A.T.
SCR	70.5/32.0	< 1%	NEG	SCR	49.4/32.2	< 1%	NEG
15 MIN	36.1/32.0	31.5%		15 MIN	36.1/32.2	35.2%	
30 MIN	36.5/32.0	28.5%		30 MIN	35.9/32.2	29.0%	
1 HR	37.2/32.0	22.9%		1 HR	37.0/32.2	24.1%	
2 HRS	38.2/32.0	22.7%		2 HRS	36.3/32.2	21.9%	
4 HRS	38.2/32.0	19.3%		4 HRS	37.6/32.2	19.4%	
6 HRS	38.5/32.0	16.5%		6 HRS	40.3/32.2	16.6%	
8 HRS	38.8/32.0	15.3%		8 HRS	39.4/32.2	15.6%	
24 HRS	44.0/32.0	5.7%		24 HRS	54.8/32.2	4.7%	
48 HRS	49.1/32.0	<1%	NEG	48 HRS	62.9/33.7	<1%	
				DSC	62.9/33.7	<1%	

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TABLE 34

FACTORATE - 01
BB 1826 -01

VITAL SIGNS

PATIENT 1

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE Per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	122/64	98.0	20	108	SCR	130/78	98.5	20	88
10 MIN	118/70	98.3	20	100	10 MIN	128/78	98.5	20	100
1 HR	120/74	98.6	20	100	1 HR	120/80	97.8	20	88
2 HRS	124/80	97.7	20	120	2 HRS	118/70	97.8	20	94
4 HRS	122/74	97.9	20	100	4 HRS	122/84	98.5	20	100

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TABLE 35

FACTORATE - 01
BB 1826 -01

VITAL SIGNS

PATIENT 2

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE Per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	112/80	98.0	20	80	SCR	120/80	98.0	20	80
10 MIN	118/74	98.0	20	88	10 MIN	120/78	97.8	20	88
1 HR	122/74	97.8	20	80	1 HR	122/80	98.0	20	84
2 HRS	112/84	97.9	20	78	2 HRS	124/78	97.6	20	88
4 HRS	120/80	98.3	20	82	4 HRS	120/78	97.6	20	88

TABLE 36

FACTORATE - 01
BB 1826 -01

VITAL SIGNS

PATIENT 3

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE Per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	110/70	99.5	20	100	SCR	118/78	98.3	20	80
10 MIN	110/80	98.7	20	88	10 MIN	112/84	98.2	20	78
1 HR	110/78	98.7	20	88	1 HR	112/80	99.0	20	80
2 HRS	122/84	99.0	20	100	2 HRS	120/80	97.8	20	80
4 HRS	122/80	99.0	20	104	4 HRS	120/80	97.6	20	120

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FACTORATE - 01
BB 1826 -01

VITAL SIGNS

PATIENT 4

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	120/84	97.5	20	64	SCR	120/70	96.9	20	80
10 MIN	118/78	97.6	20	64	10 MIN	120/70	97.6	20	80
1 HR	112/72	97.5	20	64	1 HR	120/64	97.7	20	80
2 HRS	130/70	97.1	20	64	2 HRS	130/64	97.6	20	88
4 HRS	120/74	98.3	20	64	4 HRS	118/78	98.1	20	88

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F/ RAT 01
BB 1826 -01

VITAL SIGNS

PATIENT 5

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	135/100	97.8	20	72	SCR	138/98	97.6	20	80
10 MIN	135/100	98.0	20	72	10 MIN	132/96	97.6	20	80
1 HR	134/104	97.9	20	80	1 HR	126/90	97.2	20	72
2 HRS	138/94	98.0	20	80	2 HRS	126/88	97.5	20	80
4 HRS	138/94	98.0	20	72	4 HRS	130/94	98.1	20	82

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BB 1826 -01

VITAL SIGNS

PATIENT 6

INFUSION 1					INFUSION 2				
TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min	TIME	BP mm Hg	TEMP. °F	RESP. per min	PULSE per min
SCR	118/70	98.2	20	88	SCR	120/70	97.7	20	100
10 MIN	118/80	98.2	20	88	10 MIN	122/70	98.6	20	112
1 HR	118/80	98.0	20	80	1 HR	122/78	98.2	20	80
2 HRS	120/80	97.0	20	74	2 HRS	120/80	97.6	20	100
4 HRS	130/80	98.2	20	80	4 HRS	122/80	97.5	20	120

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TABLE 40
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 1

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

TABLE 41
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 2

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

TABLE 42
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 3

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

TABLE 43
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 4

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

TABLE 44
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 5

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0		0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

TABLE 45
FACTORATE - 01
BB 1826 -01
INFUSION SITE EVALUATION

PATIENT 6

FIRST INFUSION

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

SECOND INFUISON

TIME	DISCOMFORT TYPE	DISCOMFORT SCORE	ERYTHEMA DIAMETER (mm)	ERYTHEMA SCORE	WARMTH SCORE	INDURATION DIAMETER (mm)	TENDERNESS SCORE
0	0	0	0	0	0	0	0
10 MINUTES	0	0	0	0	0	0	0
1 HOUR	0	0	0	0	0	0	0
2 HOUR(S)	0	0	0	0	0	0	0
4 HOUR(S)	0	0	0	0	0	0	0

V. MATERIALS

Revlon Health Care (U.K.) Ltd. supplied the Investigator with sufficient supplies of the following to allow for completion of the study.

Heat Treated Factorate Lot X24302H
and Lot X25203H

Two batches were selected to ensure confidence in batch reproducibility. Standard commercially available Factorate Lot Y73903 was taken from the stock at the Oxford Haemophilia Centre.

VI. PATIENT SELECTION

Four patients on a home care with documented Haemophilia A (Factor VIII deficiency) were entered into the study, employing the entry criteria below -

A) Age - 18 years or older.

B) Sex - Male.

C) Weight - 25 kg (55 lbs) or greater.

D) Health Status - Patients had documented severe Haemophilia A (Factor VIII deficiency) with baseline Factor VIII level of below 1% of normal. Patients with circulatory Factor VIII inhibitors were not entered into the study. Patients with evidence of thrombocytopenia were excluded. Patients were otherwise healthy and at work.

(a) Patient Evaluation on Entry

History of haemophilic condition (coagulation defect, severity, etc.) was noted. A complete physical examination was conducted with special attention to lymphadenopathy and splenomegaly. Pulse and blood pressure were checked.

(b) Laboratory Tests

Factor VIIIC
Haemoglobin
Haematocrit
White Blood Cell Count
Differential absolute lymphocyte count
IgG
IgM
IgA
Urea
Electrolytes
Liver Function Tests
Albumin
HBs Ag

VII. INFORMED CONSENT

Written, informed consent was obtained from all participating patients.

VIII. CLINICAL EVALUATION

(i) Study Medication and Procedures

Armour I.P. Heat Treated Factorate, a minimum of 2 batches was considered desirable for the study to ensure confidence in batch to batch uniformity. Two patients were also subsequently given Armour Standard I.P. Factorate after a washout period of at least two weeks. A pre-infusion blood sample was taken from all patients. A single dose was administered to each patient. The infusion contained 40 to 50 i.u./kg body weight and was administered over approximately 10 -15 minutes.

The number of units administered were recorded in the patient's Case Report Form.

Observation and biological sample collection for all infusions was continued over the 24 hours following the start of the infusion.

Blood was taken for laboratory studies if possible from the contra lateral arm at times zero, five minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours and 24 hours while the patients remained in the clinic.

X. RESULTS AND DISCUSSION

The results of the Factorate activity determinations for each patient are given in the summary sheets and semi-logarithmic plots made.

Patients GRO-A and GRO-A received both heat treated and non-heat treated product, hence a comparison of half-life, recovery, and time to disappearance of half initial level was possible in these patients, although for GRO-A an estimation of half-life only could be made in the case of the heat treated Factorate dose because the 24 hour level was not estimated.

Patients GRO-A and GRO-A only received the heat treated Factorate, and their 24 hour levels were not estimated, hence only calculated 'apparent' half-life could be determined by extrapolation of the 12 hour data. Recovery and time to disapperance of half the initial dose was possible in these two patients.

In those two patients where comparison of half-lives was possible, difference between the heat treated and non-heat treated product was small.

Patient GRO-A showed an apparent half-life on the heat treated Factorate of 11 hours and on the non-heat treated product of 12½ hours, while GRO-A showed an apparent half-life on the heat treated Factorate of 12 hours and 10.8 on the non-heat treated preparation.

The remaining two patients showed shorter apparent half-lives on the heat treated Factorate of 6 hours and 7 hours but these almost certainly reflect the fact that extrapolation from the 12 hour points did not truly reproduce the second slower elimination phase in the absence of a 24 hour point on the graphs.

Comparative recoveries of Factorate were as follows:

GRO-A

Heat Treated	Non-Heat Treated
2.31	1.75

GRO-A

Heat Treated	Non-Heat Treated
2.2	1.83

GRO-A

Heat Treated

1.87

GRO-A

Heat Treated

1.79

Mean recovery for Heat Treated Factorate = 2.04
Mean recovery for Non-Heat Treated Factorate = 1.79

The slightly better recovery shown for the heat treated product is almost certainly not significant.

Times to disappearance of half the initial (5 min) dose were as follows :-

GRO-A

Heat Treated	Non-Heat Treated
6.0 HRS	3.75 HRS

GRO-A

Heat Treated	Non-Heat Treated
6 HRS	6 HRS

GRO-A

Heat Treated

8 HRS

GRO-A

Heat Treated

4.25 HRS

No abnormalities were shown in the biochemistry or haematology, other than those expected from the patients' haemophilia. Heart rates and blood pressures were normal. None of these parameters were altered at the completion of the study. Full results of laboratory tests are given in the appendix to this report.

AP000571

XI. ADVERSE REACTIONS AND SIDE EFFECTS

None were observed during this study.

XII. CONCLUSIONS

A small comparative study of Heat Treated Factorate against the standard non-heat treated product was completed in four severe haemophiliac patients. The half-life of the second phase in both heat treated and non-heat treated Factorate was essentially similar, as was the recovery and the time to reduce to half initial Factor VIII levels.

It may therefore be concluded that heat treatment does not affect these in-vivo aspects of the product.

No adverse reactions or local intolerance to infusions were encountered.

SUMMARY SHEET - FACTORATE KINETIC STUDY

Patient - GRO-A

Weight - 68 Kg

Batch of Factorate - NON-HEAT TREATED - Y73903

Dose Volume - 176 ml

Dose Potency - 19.4 u/ml

Total Units given - 3414

Factorate assay (units/decilitre)

Time	Assay Value
Pre	< 1
5 mins post-dose	92
30 mins post-dose	90
1 hour post-dose	80
2 hours post-dose	68
4 hours post-dose	50
6 hours post-dose	47
12 hours post-dose	24
24 hours post-dose	12

RESULTS OF LABORATORY TESTS ON PATIENTS PARTICIPATING IN FACTORATE HEAT TREATED KINETIC STUDY

PATIENT INITIALS: GRO-A

TEST	PRE	END OF STUDY
Hb (g/100 ml)	14.8	14.9
RBC (x 10^{12}/l)	4.89	4.94
WBC (x 10^{9}/l)	4.8	5.02
Diff.	$P_{47}L_{43}M_{6}E_{4}$	$P_{49}L_{40}M_{5}E_{5}B_{1}$
Absolute Lymph (x 10^{9}/l)	2.06	-
Platelets (x 10^{9}/l)	-	2.06
Sodium (mmol/l)	143	142
Potassium (mmol/l)	3.7	3.5
Chloride (mmol/l)	98	96
Bicarbonate (mmol/l)	26	19
Urea (mmol/l)	4.1	4.8
Creatinine (µmol/l)	88	-
Total protein (g/l)	72	73
Albumin (g/l)	47	44
Total bilirubin (µmol/l)	11	11
AST (iu/l)	31	29
G-Gt (iu/l)	118	125
Alk. Phos. (iu/l)	233	230
HBs Ag	Not detected	Not detected
Anti-HBs Antibody	Positive	Positive
IgG (g/l)	11.5	11.8
IgA (g/l)	1.8	1.9
IgM (g/l)	1.2	1.1
Electrophoresis	Normal	Normal

AP000575

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RESULTS OF LABORATORY TESTS ON PATIENTS PARTICIPATING IN FACTORATE HEAT TREATED KINETIC STUDY

PATIENT INITIALS: GRO-A

TEST	PRE	END OF STUDY
Hb (g/100 ml)	15.2	15.2
RBC (x 10^{12}/1)	4.96	4.95
WBC (x 10^{9}/1)	5.5	5.31
Diff.	-	-
Absolute Lymph (x 10^{9}/1)	-	2.13
Platelets (x 10^{9}/1)	235	207
Sodium (mmol/1)	141	141
Potassium (mmol/1)	3.6	4.0
Chloride (mmol/1)	101	103
Bicarbonate (mmol/1)	20	20
Urea (mmol/1)	5.5	5.9
Creatinine (µmol/1)	51	84
Total protein (g/1)	75	75
Albumin (g/1)	42	45
Total bilirubin (µmol/1)	21	14
AST (iu/1)	36	32
G-GT (iu/1)	16	18
Alk. Phos. (iu/1)	283	264
HBs Ag	Not detected	Not detected
Anti-HBs Antibody	Positive	Positive
IgG (g/1)	Not done	Not done
IgA (g/1)	Not done	Not done
IgM (g/1)	Not done	Not done
Electrophoresis	Not done	Not done

AP000576

RESULTS OF LABORATORY TESTS ON PATIENTS PARTICIPATING IN FACTORATE HEAT TREATED KINETIC STUDY

PATIENT INITIALS: GRO-A

TEST	PRE	END OF STUDY
Hb (g/100 ml)	14.5	14.2
RBC (x 10^{12}/1)	5.1	4.90
WBC (x 10^{9}/1)	4.7	5.92
Diff.	-	$P_{64}L_{24}M_{9}E_{2}B_{1}$
Absolute Lymph (x 10^{9}/1)	1.2	1.40
Platelets (x 10^{9}/1)		
Sodium (mmol/1)	139	139
Potassium (mmol/1)	4.2	3.9
Chloride (mmol/1)	101	101
Bicarbonate (mmol/1)	25	26
Urea (mmol/1)	3.4	3.6
Creatinine (µmmol/1)	69	77
Total protein (g/1)	78	78
Albumin (g/1)	43	42
Total bilirubin (µmol/1)	7	6
AST (iu/1)	97	78
G-GT (iu/1)	37	39
Alk. Phos. (iu/1)	244	250
HBs Ag	Not detected	Not detected
Anti-HBs Antibody	Positive	Positive
IgG (g/1)	21.7	20.7
IgA (g/1)	2.6	2.4
IgM (g/1)	2.5	2.5
Electrophoresis	Normal	Normal

AP000577

ARMOUR001570

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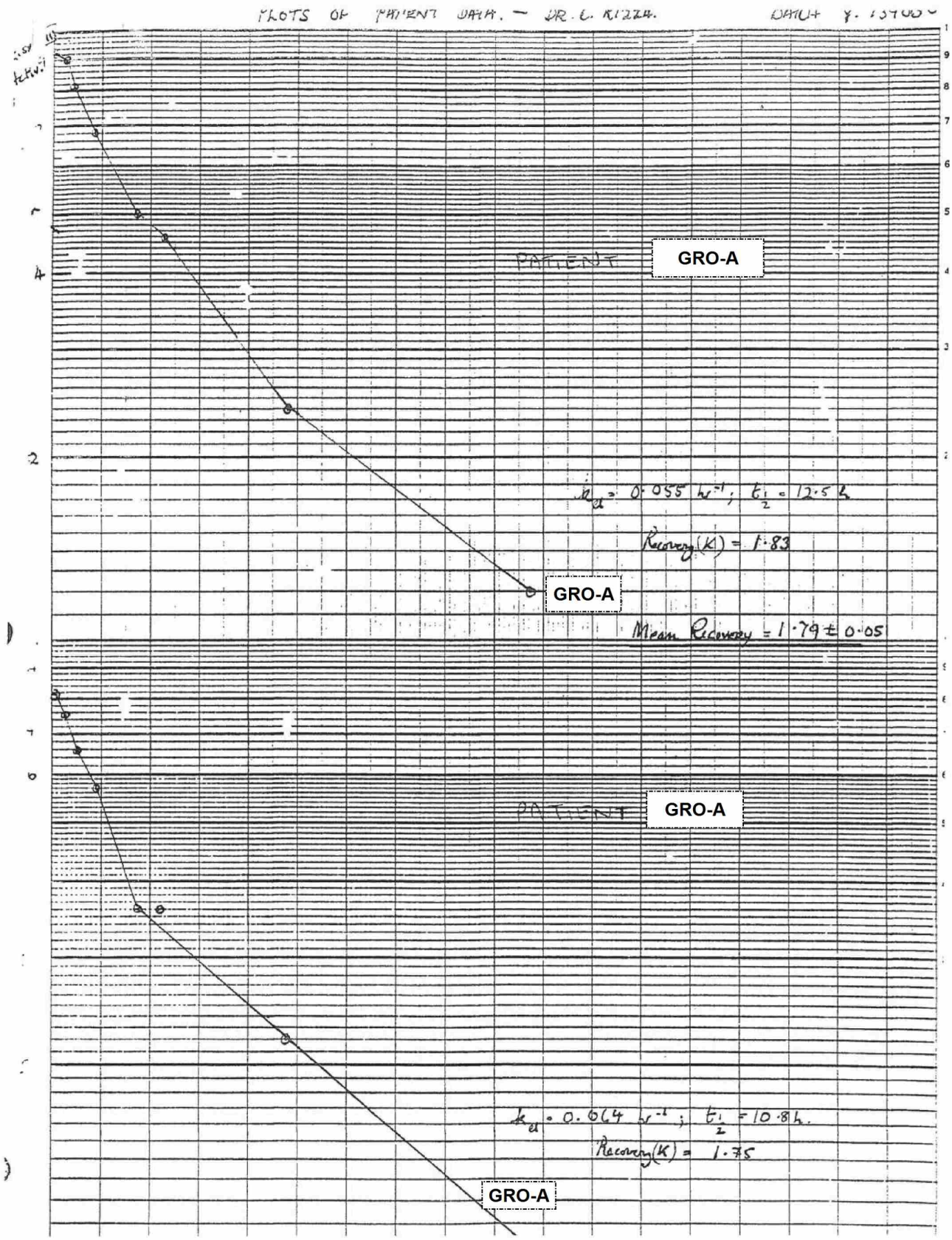
RESULTS OF LABORATORY TESTS ON PATIENTS PARTICIPATING IN FACTORATE HEAT TREATED KINETIC STUDY

PATIENT INITIALS: GRO-A

TEST	PRE	END OF STUDY
Hb (g/100 ml)	13.9	13.9
RBC (x 10^{12}/l)	4.76	4.76
WBC (x 10^{9}/l)	3.8	4.2
Diff.		
Platelets (x 10^{9}/l)	173	166
Absolute Lymph (x 10^{9}/l)	1.56	1.8
Sodium (mmol/l)	142	141
Potassium (mmol/l)	3.5	3.6
Chloride (mmol/l)	102	97
Bicarbonate (mmol/l)	28	25
Urea (mmol/l)	3.4	4.7
Creatinine (µmol/l)	81	72
Total protein (g/l)	75	79
Albumin (g/l)	42	44
Total bilirubin (µmol/l)	32	34
AST (iu/l)	45	47
G-GT (iu/l)	14	14
Alk. Phos. (iu/l)	209	210
HBs Ag	- ve	Not detected
Anti-HBs Antibody	+ ve	
IgG (g/l)	17.3	23.1
IgA (g/l)	2.3	3.1
IgM (g/l)	1.3	1.2
Electrophoresis	Slight hyper 8	Slight hyper 8

AP000578



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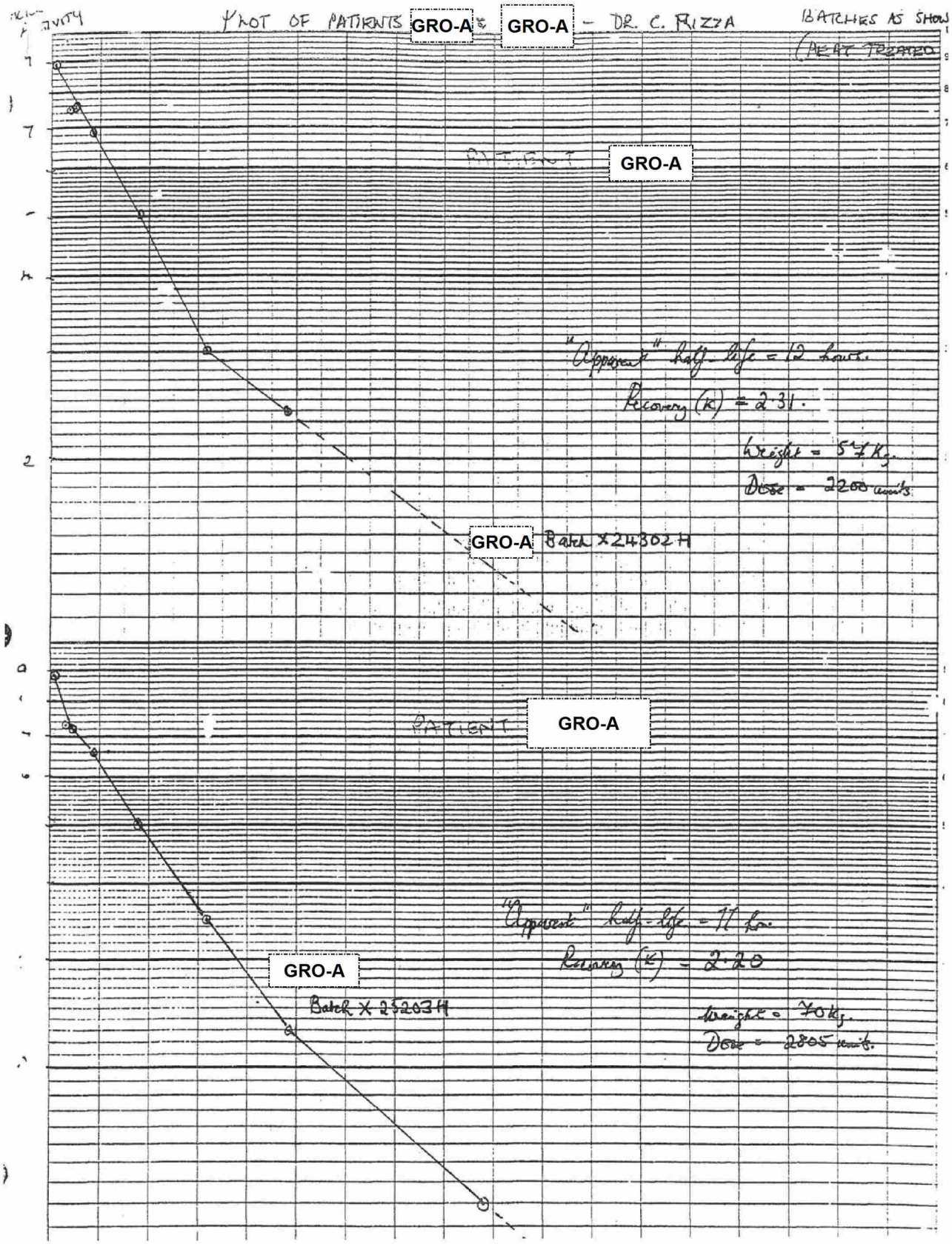

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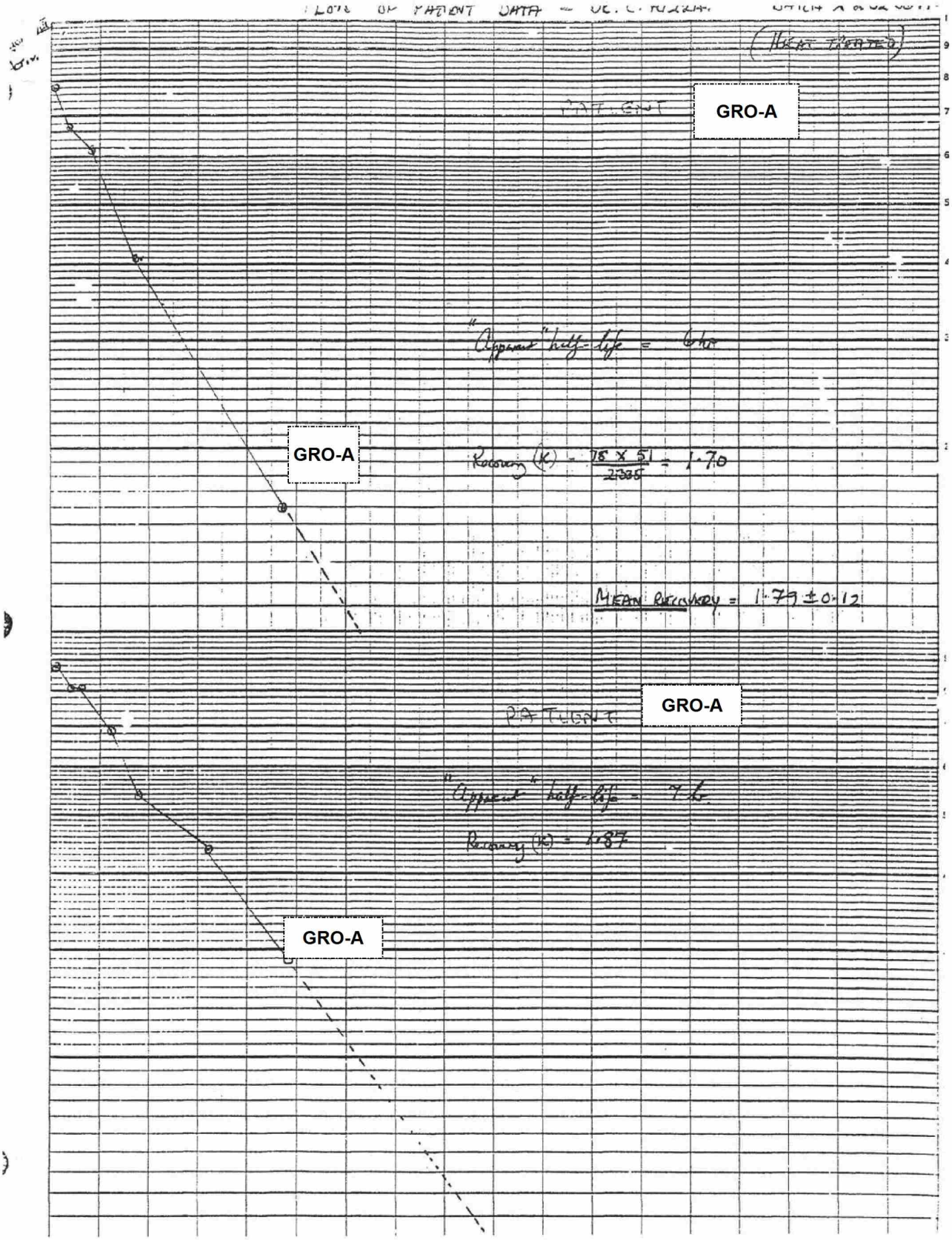

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