PA10/6/1-3

FACTORATE

(Heat-treated)

Supportive Data for Clinical Investigation.

January 1984.

PROTOCOL FOR THE STUDY OF

HEAT-TREATED FACTORATE (FACTORATE H.T.)

IN LONG TERM TREATMENT OF HAEMOPHILIA

PROTOCOL VIII - 201

Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne
East Sussex

May, 1983

HAT/EB

INTRODUCTION

One of the primary concerns in the use of coagulation factors in the haemophiliac patient who has mild to moderate disease which requires infrequent treatment or in the newly diagnosed patient, is the knowledge that each exposure presents a risk of causing hepatitis.

An intense effort to reduce the hepatitis infectivity of coagulation factors has been ongoing. All donors are now screened to eliminate identifiable hepatitis carriers. Currently, process modifications are being implemented to reduce infectivity of coagulation proteins. Our current approach is to treat with heat the Factor VIII Concentrate and although animal studies have shown reduced infectivity the ultimate test is the use of the new product in patients requiring coagulant therapy to maintain hemostasis or to end a bleeding episode.

OBJECTIVE

It is the purpose of this study to use our specially prepared Factorate product exclusively for an extended period of time in a number of previously untreated patients or in those who have received minimal treatment to determine if infectivity of the product has been elir .inated.

Minimal treatment is defined as having received no Concentrate or cryo-precipitate during the preceeding six (6) months and not having undergone major surgery requiring large amounts of blood and blood products at one time during the preceeding three (3) years and having no history of hepatitis, yellow jaundice, sub-clinical hepatitis or any abnormal liver function tests.

DEFINITION OF HEPATITIS

A patient will be considered to be suffering from acute hepatitis if he develops clinical symptoms and signs or shows an increase of at least two and a half times the upper limit of normal serum aminotransferase levels, having had normal values previously.

Hepatitis will be classified as acute icteric (raised serum bilirubin)

... anicteric

... symptomless

This may be of two varieties - hepatitis B or non-A, non-B. Hepatitis A, cytomegalovirus infection, glandular fever and toxoplasmosis will be excluded by appropriate laboratory tests.

STUDY DESIGN

Selected study sites will be haemophilia centres run by recognized experts in haemophilia care, who have an adequate number of patients to assure the recruitment of at least five (5) untreated subjects each over a one (1) year study period. In addition, these centres will be asked to recruit an equal number of patients who have had infrequent treatment and are free of hepatitis markers. These markers include hepatitis-B surface antigen, antibody to surface antigen, and antibody to core. They will also have normal liver function studies and have no history of hepatitis.

It will be essential for the centres recruited to have close control over their patients to ensure that those entered into the study have access to and use only the trial Factorate. Any break .n this rule will end the study for that subject at the time the non-study product is used. Patients will be entered into the study as they require Factorate H.T.

The end point of this study will be the presence or absence of hepatitis as measured by hepatitis markers and liver chemistries taken serially over the one year period of study.

The final study design will be consistent with the study centre medical and administrative management procedures. Every attempt will be made to have the study design fit smoothly into already-established study centre practices. Any deviation from the protocol will be reported to the study monitor by telephone. A written report should be sent promptly following the call. Should it be necessary to drop a patient from the study a determination of the length of follow-up will be made based on the dose and duration on protocol study and the reason for termination.

All patients or their guardians will have the purpose of the study carefully explained and will sign an Informed Consent. They will understand and agree to the use of study Factorate exclusively for the one year period of the study. However, in the best interests of their patients, the physicians may prescribe any treatment considered necessary. If this includes Factor VIII other than the study material the patients will continue to be followed but not included in the analysis.

PLAN OF STUDY

1. Entry Criteria - Group A

   a) Diagnosis of Haemophilia A established by Factor VIII C levels < 20%, with evidence of bleeding.

   b) No history of use of any blood products.

   c) Normal liver function studies.

   d) Negative hepatitis-B markers.

   e) No history of hepatitis - clinical or sub-clinical.

   f) An attempt will be made to screen immediate family contacts before entry into the study in order to include only those where there is no history or laboratory evidence of hepatitis. The tests will include serology for hepatitis A & B and liver function tests.

2. Entry Criteria - Group B

   a) Diagnosis of Haemophilia A established by Factor VIII C levels < 20% of normal with evidence of prolonged bleeding.

   b) History of infrequent use of cryo-precipitate or concentrate (no use in past six (6) months). No history of use of blood products other than Factor VIII for surgery or trauma in past three (3) years.

   c) No evidence of hepatitis markers.

   d) Normal liver function studies.

3. Exclusion Criteria

a) Any hepatic abnormality.

b) Other disabling diseases which would interfere with objectives of the study. This could include those with known defects which may require packed red cells or whole blood or any serious illness.

c) A member of a transient family group.

d) Parents, guardians, or adult patients with limited intelligence and difficulty understanding or accepting the restrictions of this study should be excluded.

4. Study Design

prior to entering the study, a history, including details of previous transfusions, will be recorded, a complete physical examination performed, and blood collected for baseline laboratory studies. These will include a full blood count, liver function tests and hepatitis A & B antibody.

If the patient is seen as an emergency, then as many tests will be performed as is compatible with the situation.

Each patient entered into the study will agree to use only Factorate H.T. for treatment or prophylaxis of any bleeds from whatever cause. A careful explanation will be given to the parent, guardian, or responsible patient of all risks and benefits they are taking in agreeing to this one (1) year period of study. Informed Consent will be signed.

The patients will be mild haemophiliacs and therefore not on home treatment. Any intercurrent illness will be recorded with date and time of such illness. Instructions on maintaining telephone contact with the investigator will be given with reasons for making contact.

The patient records or duplicates will be segregated at the treating institution to ensure that the investigator or staff will be notified at any time the patient enters the institution either as an out-patient or in-patient.

In the absence of transfusion hepatitis patients will be followed for 1 year following treatment with heat treated Factorate. Liver function tests and tests for hepatitis A & B markers, CMV & EBV will be carried out at appropriate intervals. Blood will be collected pre-treatment and at weeks 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 40 and 52 post-transfusion. In the event of the patient developing evidence of acute hepatitis, his liver function tests and hepatitis B serology will be followed fortnightly until his condition resolves or for 3 months after the onset and if his condition has not resolved then monthly for 6 months. Follow-up after this will be 3 monthly for the next 3 years.

This study will continue for twelve months. A complete physical examination will be repeated at the twelve month visit.

Those patients whose liver function tests remain elevated for one year after the attack of non-A, non-B hepatitis or become carriers of hepatitis B virus will be referred to the local liver clinic for investigation of chronic liver disease. Liver biopsy will only be carried out if clinically indicated.

LABORATORY TESTS

The basic tests will be conducted at the chosen local Haemophilia Centres. In addition, it is proposed that sera from patients who have received heat-treated Factorate should be made available to the Hepatitis Working Party for use when tests for non-A, non-B hepatitis become available. A 2.0 ml aliquot serum obtained in the follow-up period will be sent to Dr. Craske at the Public Health Laboratory, Withington Hospital, Manchester, M20 8LR for this purpose.

(a) Tests performed prior to study and at 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 40 and 52 weeks post-transfusion to include :-

1) Haematocrit, Haemoglobin, white blood count and differential count.
2) Absolute lymphocyte count, percent T cells and B cells) if locally
3) Factor VIII-C and Factor VIII ag, IgG, IgM, IgA) available
4) Urine analysis including microscopic examination of the sediment.
5) Blood creatinine.
6) Hepatitis screen to include Hepatitis-B surface antigen, hepatitis-B surface antibody, hepatitis-B core antibody.
7) CMV and EBV.

8) Liver enzymes to include SGOT, SGPT, and alkaline phosphatase. Abnormal values will be repeated to confirm the tests.

(b) Liver biopsy only if indicated.

DRUG SUPPLIES

The investigator will be provided with adequate supplies of Factorate H.T. for each patient enrolled. These supplies should be refrigerated and specifically assigned to the enrolled subjects. Provisions must be made to prevent the inadvertent substitution of other products, whether from Armour or other manufacturers, to replace any of the study material. As each vial is assigned, a record of the lot number and quantity will be recorded on the subject's record and case report form. If the supplies held by the investigator become exhausted before adequate replacement can be effected, emergency supplies may be obtained by telephoning one of the three nominated persons designated at the back of this protocol.

Those vials assigned to home care will likewise be recorded on the subject's report form, as well as on the patient's self-kept record of product use. Patients should confirm the presence of adequate refrigerated storage at home.

The dose of Factorate will be determined by the investigator based on the severity of the bleed and experience in treating patients. As a general rule, 1 unit of AHF activity per Kg of body weight, will increase plasma circulating AHF levels by 2%.

Infusion rates of the reconstituted product should be adjusted to a rate comfortable to the patient about 2 ml per minute.

ADVERSE EFFECTS

Any untoward reaction to the infusion of Factorate H.T. should be reported to the investigator who in turn will maintain a record in the case report form.

Abnormal laboratory values should be reported to the sponsor by telephone as soon as practical. Severe reactions should be reported immediately to the sponsor. Written reports will follow the telephone reports.

ANALYSIS OF DATA

The end point to be analysed is the presence or absence of hepatitis as manifested by serial liver enzymes and hepatitis markers.

All reported side effects will be listed and tabulated.

Laboratory data will be summarized.

Clinical control of bleeding will be evaluated.

DOCUMENTATION

Signed Informed Consent which conforms to the guidelines of the Food and Drug Administration will be obtained by the investigator.

Institutional Review Board approval will be submitted to the sponsor prior to the study.

Case Report Forms and Patient Report Cards, provided by the sponsor, will be completed and submitted at the end of the study.

WITHDRAWAL FROM FURTHER TREATMENT WITH HEAT-TREATED FACTORATE

1. Will occur on request of patient or guardian.

2. Patients unable to follow the protocol.

3. Patients whose liver function studies become abnormal if the abnormality is due to Factorate. Repeat testing will be done to exclude an aberrant value and every effort will be made to exclude any other causative agent.

4. Patients whose hepatitis markers become positive. Family contacts will be screened to exclude them as a source of infection.

5. Patients receiving whole blood or blood products other than study drug.

Follow-up of Subjects:

Every effort will be made to follow every subject who has received at least one treatment with heat-treated Factorate. This will be for a full year as set out above or more frequently or in greater depth if clinically indicated.

Only those patients who, in the course of the one year study period have received whole blood, blood products other than the study material or, who have family contacts proven to have developed hepatitis and/or a source of infection will be excluded from the analysis.

Those patients requiring a second dose of Factor VIII will, as far as is clinically reasonable, receive heat-treated Factorate.

REPORTING OF SIDE EFFECTS

All side effects and abnormal laboratory values to be reported by telephone as soon as possible to:

Dr. H. L. Shaw
Medical and Technical Director
Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne

Tel. Day: Eastbourne GRO-C
Night: Brighton GRO-C

or

Mr. R. B. Christie
Director of Clinical Sciences
Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne

Tel. Day: Eastbourne GRO-C
Night: Eastbourne GRO-C

EMERGENCY SUPPLIES

If fresh supplies of Heat Treated Factorate are required they may be obtained at any time by telephoning one of the persons indicated below:

Mr. R. B. Christie
Director of Clinical Sciences
Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne

Tel. Day: Eastbourne GRO-C Ext. GRO-C
Night and Weekends: Eastbourne GRO-C

or

Mr. D. Cockayne
Head of Pharmaceutical Sciences
Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne

Tel. Day: Eastbourne GRO-C Ext. GRO-C
Night and Weekends: Eastbourne GRO-C

or

Mr. S. G. Brooks
Clinical Research Associate
Revlon Health Care (U.K.) Limited
St. Leonard's House
St. Leonard's Road
Eastbourne

Tel. Day: Eastbourne GRO-C Ext. GRO-C
Night and Weekends: Eastbourne GRO-C

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

AP000262

ARMOUR001153

FACTORATE HEAT TREATED BATCHES - STABILITY DATA

Batch Number X23102-H

Initial Oxford assay - 198iu/vial

	Potency (iu/vial)	% Initial Potency	% Label Potency
3 Months, 4°C Storage	197	99.5	98.5
3 Months, 37°C Storage	190	96.0	95.0
1 Month, 50°C Storage	183	92.4	91.5
2 Months, 50°C Storage	181	91.4	90.5
3 Months, 50°C Storage	179	90.4	89.5

Batch Number X24302-H

Initial Oxford assay - 218iu/vial

	Potency (iu/vial)	% Initial Potency	% Label Potency
3 Months, 4°C Storage	195	89.5	97.5
3 Months, 37°C Storage	199	91.3	99.5
1 Month, 50°C Storage	197	90.4	98.5
2 Months, 50°C Storage	188	86.2	94.0
3 Months, 50°C Storage	171	78.4	85.5

Batch Number X25203-H

Initial Oxford assay - 213iu/vial

	Potency (iu/vial)	% Initial Potency	% Label Potency
3 Months, 4°C Storage	191	89.7	95.5
3 Months, 37°C Storage	197	92.5	98.5
1 Month, 50°C Storage	200	93.9	100.0
2 Months, 50°C Storage	182	85.4	91.0
3 Months, 50°C Storage	168	78.9	84.0

REVLON HEALTH CARE GROUP
RESEARCH AND DEVELOPMENT DIVISION

DEPARTMENT: PROTEIN BIOCHEMISTRY

DIVISION: PLASMA FRACTIONS

THE IN VIVO INFECTIVITY ASSAY OF HEAT-TREATED ANTIHEMOPHILIC FACTOR CONTAINING NON-A, NON-B HEPATITIS VIRUS (HUTCHINSON STRAIN)

STUDY REPORT NO. PFR 83-003

LOCATION OF INVESTIGATION:	Southwest Foundation For Research & Education
MANAGING INVESTIGATION:	Jorge Eichberg D.V.M., Ph.D.
UNCOMPENSATED CONSULTANT:	Robert H. Purcell, M.D.
SPONSOR:	Revlon Health Care Group 1 Scarsdale Road Tuckahoe, NY 10707
STUDY DIRECTOR:	Micheal E. Hrinda, Ph.D.
REPORT PREPARED BY:	Michael E. Hrinda, Ph.D.

GRO-C

Michael E. Hrinda, Ph.D.
Director, Protein Biochemistry

DATE June 14, 1983

APPROVED BY:

GRO-C

R.H. Landaburu, Ph.D.
Director, Division of
Plasma Fractions

DATE 6-16-83

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THE IN VIVO INFECTIVITY ASSAY OF HEAT-TREATED ANTIHEMOPHILIC FACT(
CONTAINING NON-A, NON-B HEPATITIS VIRUS (HUTCHINSON STRAIN)

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I. TITLE

"The In Vivo Infectivity Assay of Heat-Treated Antihemophilic Factor Containing Non-A, Non-B Hepatitis Virus (Hutchinson Strain)"

II. BACKGROUND

In preliminary experiments, Revlon Health Care scientists had tested a process methodology which, if efficaceous, could increase the safety of antihemophilic factor or other Human Blood Component products by reducing the infectivity of hepatitis virus(es) which could be undetectable by in vitro test methods. The process selected was the application of heat at 60°C for a time period of 30 hours to lyophilized commercial Factor VIII concentrate (Factorate^{R}, Armour). Chemical tests and biologic potency tests demonstrated that the heating process altered the products minimally or undectectably, and it was decided to support further development of the procedure with in vivo efficacy testing. The test chosen was the chimpanzee model of hepatitis infection; test products were intentionally infected with a challege dose of Hepatitis B virus (MS-2 pool, strain ayw) at a level (3000 CID_{50} per chimpanzee) which has been previously demonstrated to give a high incidence and rapid onset of infection. The experimental product

was tested in three chimpanzees, one receiving unheated AHF with virus as a positive control. The results of this preliminary efficacy test conclusively demonstrated that heating lyophilized AHF at 60°C for 30 hours did not diminish the infective potential of the Hepatitis B challenge virus. It was noted, however, that the positive control animal experienced two courses of disease, the first of which was concluded due to a Non-A, Non-B agent, presumed for this reason to be present in the test substance. Since the two experimental animals did not experience infection with the Non-A, Non-B agent, a further preliminary conclusion was drawn that the heating process rendered this agent non-infective in this experiment. The lack of knowledge of the titer of the accidental NANB agent, or its identity, did not allow this conclusion to be made firmer. The present study was designed in order to test this tentative conclusion with a known Non-A, Non-B virus (NANBV) under controlled circumstance.

III. OBJECTIVE

The objective of this experiment was to compare the infectivity of heat-treated Factorate^{R} (Armour) intentionally infected with NANBV to that of an identical batch of infected Factorate^{R} (Armour) which was not heat treated, in the chimpanzee model of NANB hepatitis infection.

IV. INVESTIGATORS

Michael E. Hrinda, Ph.D. - Study Director
Director of Protein Biochemistry
Revlon Health Care R & D

Robert H. Purcell, M.D. - Uncompensated Consultant
Chief, Hepatitis Virus Section
Laboratory on Infectious Disease
NIAID
NIH Bldg. 7, Room 202
Bethesda, Maryland 20205

S. Kalter, Ph.D. - Senior Consultant
Jorge Eichberg D.V.M., Ph.D. - Managing Scientist
Southwest Foundation for Research & Education
P.O. Box 28147
San Antonio, Texas 78284

V. LOCATION OF EXPERIMENT

The experiment was conducted in the primate facilities of:

The Southwest Foundation For Research & Education
P.O. Box 28147
W. Loop 410 At Military Dr.
San Antonio, Texas 78284

VI. STUDY DESIGN

This was an open study requiring 5 healthy chimpanzees which were each inoculated with identical (other than the heat treatment) vials of Factorate^{R} (Armour) after initial baseline observation. The vials received by each animal contained 3000 CHD of NANBV. Two animals received vials which were not heated (positive controls) and three animals received vials which had been heated (experimentals). Three of the animals, one an unresponsive positive control and two experimentals, which had not

contracted disease for 22 weeks post-inoculation, were challenged with an additional inoculation of 3000 CHD of NANBV in saline vehicle. The observation period for all animals was continued until any disease onset had abated by serologic criterea, or, in the case of animals which remained disease free by these criterea, for 6 months after the last inoculum was injected. This is summarized below in tabular form.

	INITIAL INOCULUM		CHALLENGE AT 22 WEEKS	TOTAL OBSERVATION PERIOD (WEEKS)
ANIMAL #	Heated	Not Heated	Not Heated	
4-58 (PETE)	-	Yes	Yes	47
4x38 (FREDA)	-	Yes	No	25
4x62 (BERT)	Yes	-	Yes	44
4x36 (Lisa)	Yes	-	Yes	44
4x50 (Elaine)	Yes	-	No	26

VII. PREPARATION OF INOCULA

A. Viral Strain Used for Inocula: NANB Virus Infective Pool, Hutchinson strain, 10^{-2} dilution, NIAID stores dated 7-12-77

B. Test Material: Vials of Factorate^{R} Generation II, a commercially released lot #V44106, containing a nominal 1000 AHF units (to be reconstituted at infusion with sterile water for Injection, U.S.P. 30 ml.) were obtained from Armour Pharmaceutical Co., Kankakee, Illinois.

C. Method of Preparation, Primary Inocula:
(All preparatory activities except as indicated were conducted in a Biohazard safe environment at the Meloy

Laboratories, Springfield, VA)

(1) 10 vials of Factorate^{R} were reconstituted with WFI as directed on package insert.

(2) The contents of the 10 vials were removed and pooled into a sterile glass beaker. The pool was mixed and non-viral infected aliquots removed to new clean sterile lyophilization vials.

(3) To the remaining pool of 6 x 30 ml (nominal) of AHF solution was added 2 ml of Hutchinson pool virus (10^{-2} dilution) giving a final dilution of the virus of 10^{-4}. (Each vial of F.VIII after reconstituion with 30ml of WFI occupies slightly more than 30ml due to the solids volume in the vial)

(4) The infected pool was mixed and 30 ml aliquots were removed to new clean sterile lyophilization vials.

(5) All vials were frozen lyophilized and sealed.

(6) Vials with disinfected exterior surfaces were transferred to the sponsor's laboratories.

(7) Experimental vials were heat treated by total immersion in a regulated laboratory water bath in the sponsor's laboratories. Immersion time and temperature were recorded by placing a calibrated thermocouple into the lyophilized cake of a similar vial of Factorate^{R}, then evacuating and sealing the vial. This recorder

probe ("dummy vial") was used to indicate temperature in the experimental vials. Heating time was measured to be in excess of 30 hours after the product temperature had reached and held 60°C ± 0.5C.

D. Method of Preparation, Challenge Inocula:

The primary inocula above consisted of 30 ml per animal of a 10^{-4} dilution of Hutchinson pool virus, or a 1/100 dilution of the 10^{-2} NIAID stock solution. For the challenge inocula, 1 ml of solution containing the same quantitiy of virus was prepared. 1/100 (dilution in primary inocula) x 30 ml = 30/100 = 3/10 = 1/3.3. 1 ml of the 10^{-2} NIAID stock was diluted with sterile saline to 3.3 ml. This inocula was stored as a single stock solution for multiple injection of 1 ml each. This stock solution was injected into 3 chimpanzees.

VIII. SELECTION OF EXPERIMENTAL SUBJECTS

Adult chimpanzees (Pan troglodytes) were selected by the managing scientist from the colony at the Southwest Foundation, hereafter SFRE. The chimpanzees were required, in specific, to be free of any evidence, clinical or serologic, of hepatic disease or disorder at the initiation of the experiment. This was confirmed by measurement of serum enzyme levels during baseline observation. The enzymes measured were ALT (SGPT), AST (SGOT), AND GGT. All five animals were known to have positive antibody titers against

Hepatitis B surface antigen; in two cases this was due to prior inoculation with experimental vaccines to the Hepatitis B agent. This was considered suitable for the present study as the investigators believed this could be a deterent to the appearance of undesired non-specific infection with HBV in any of the animals. Because of a shortage of animals which could be selected as candidates for this study, it was decided to include an animal, 4-58, which had been suspected of infection with tuberculosis 6 months prior to the present study. The infection was not verified in continued testing, but 4-58 was placed on Rifanate^{R} (Rifampin-Isoniazid) therapy 4 months prior to the present study and this therapy continued for 9 months duration. The use of the animal was predicated on the lack of signs of non-specific hepatitis which was drug associated and that the animal was selected to be a positive control, rather than an experimental subject. As discussed in the results, it is interesting to note that 4-58 remained resistant to infection with NANBV after 2 inoculations with 3000 CHD each. It cannot be determined if this is drug associated. The study panel was:

SUBJECT	AGE	SEX	WT. (kg)	POSITION IN PRES. STUDY
4-58 (PETE)	10	M	45	POS. CONT.
4x38 (FREDA)	8	F	19	POS. CONT.
4x62 (BERT)	5	M	20	EXPER.
4x50 (ELAINE)	6	F	24	EXPER.
4x36 (LISA)	≈13	F	42	EXPER.

IX. CONDITIONS OF THE EXPERIMENT

All participating scientists were notified in the acceptance of a written protocol for the experiment that this efficacy study was nonetheless to be performed so as to follow established guidelines for the conduct of animal experimentation by the regulations of Good Laboratory Practices. The Study Director was informed in writing by Dr. William J. Goodwin, Chairman of the SFRE Quality Assurance Committee, that a Quality Assurance unit was in operation at SFRE.

The study panel used in the experiment was housed in isolation from the remainder of the SFRE colony. Two isolation units were employed. One for animals which had received known positive inocula, the other for the experimental animals.

Deviations from the written protocol were recorded by the Study Director. Separate written protocols were issued and used for inocula preparation. Confirmatary cross-identification of animals and inocula was transmitted to the Study Director at the time of each inoculation. Copies of the original work slips for laboratory tests were received by the Study Director on a weekly basis. Reserve serum samples from all testing performed are held both at SFRE and by the Study Director.

X. EXPERIMENTAL PROTOCOL

A. Pre-Inoculation Studies -

Serum AST (SGOT) - weekly
" ALT (SGPT) - weekly
" GGT - weekly
" AUSRIA - at baseline initiation or monthly thereafter
" AUSAB - " "
" HAVAB - " "
" CORAB - " "
Percuntaneous Punch Liver Biopsy - 1 week prior to inoculation

B. Day of Inoculation

Serum AST
" ALT
" GGT
" AUSRIA
" AUSAB
" HAVAB
" CORAB
Percutaneous Punch Liver Biopsy

C. Post-Inoculation Studies

Serum AST - weekly
" ALT - "
" GGT - "
" AUSRIA - monthly
" AUSAB - monthly
" HAVAB - "
" CORAB - "
Percutaneous Punch Liver Biopsy - monthly

D. General Observations

Complete Blood Count - Bi-monthly

Complete Physical Exam on SFRE annual schedule.

E. Processing & Test Performance

Blood Chemistries were performed by the SFRE Laboratories.

Liver biopsy specimens were processed in buffered formalin by the SFRE Laboratories, and samples were sent to a further laboratory for processing and mounting slides for contigency light microscopic examination pending electron microscopic results. Samples of biopsy specimen fixed in glutaraldehyde were studied by Dr. G. Con Smith, III at SFRE and a report submitted to the Study Director.

F. Observation Standards

The standard accepted for an elevation of serum enzymes was twice the baseline value in each animal observed. The standards used by Dr. Con Smith III in his electron microscopic examination are noted in his summary report (Exhibit A).

XI. RESULTS & DISCUSSION

A. Positive Control Response - Unheated Test Product

The positive control animals were 4x38 and 4-58. Each animal received as a primary inoculum the entire content of an unheated vial of Factorate^{R} which contained, per vial, 3000 CHD of NANBV. The baseline ALT of animal 4x38 was 18.9 $\pm$ 2.89 I.U./L. The ALT was marginally and non-significantly elevated in the first week post inoculation and increased steadily to more than twice baseline (45.2 I.U./L) by week 7 post inoculation. Peak

ALT levels of 193 IU/L were reached at week 12 post inoculation. The level returned to within twice the baseline value by week 16 and did not significantly change through the remainder of the 24 week observation. Similar patternswere observed with the other two enzymes, AST levels less markedly elevated and GGT levels more strongly elevated in relation to baseline than the ALT. (See table I). Electron microscopic examination of histologic specimens were negative at the time of inoculation and at 4 weeks. They were strongly positive for structures indicative of NANBV infection at weeks 8 and 12, reduced at week 16 and normal in appearance by week 16 (Table I, Exhibit A).

The baseline ALT of animal 4-58 was 19.0 $\pm$ 4.06. No significant changes in ALT, AST or GGT were noted in 22 weeks observation post inoculation (Table II). The animal was therefore inoculated at weeek 23 with an additional dose of 3000 CHD of the same virus diluted in saline rather than in AHF test product. No positive serologic sign of hepatitis was noted in an additional observation of 24 weeks following this challenge dose. Subsequent examination of histologic specimens failed to detect the presence of cytoplasmic tubules characteristic of NANBV infection at all sampled points.

We believe the evidence indicates that animal 4x38 became infected with NANBV in a time frame characteristic

of this dosage of the Hutchinson strain virus, and that the infection of this animal validates the infectivity of the experimental product prior to being subject to the heating process. We conclude animal 4-58 to be completely resistant to infection with NANBV of the Hutchinson strain since it not only failed to respond to the primary inoculum but also to a secondary challege with an inoculum the infectivity of which was validated in two other animals (see below).

B. Experimental Response - Heated Test Product

Three animals, 4x62, 4x36, 4x50, were each inoculated with a test vial of Factorate[R] which had been heated. There were no observed changes in any of the three serum enzymes (AST, ALT, GGT) as a result of this incculation (Tables III, IV, V). Animal 4x50 was followed for 26 weeks post-inoculation before termination of the experimental observation (Table III). After 22 weeks of negative serologic evidence of infection in animals 4x62 and 4x36, they were each on week 23 inoculated with a challenge dose of 3000 CHD of the Hutchinson virus in saline vehicle to demonstrate the susceptibility of these test animals to NANB infection. The ALT level in 4x62 rose to a peak level of 153.1 I.U./L. in the seventh week post challenge; a similar rise in both AST and GGT accompanied this response. The ALT level was elevated by the 3rd and 4th week post

challenge but all values prior to the peak elevation were within twice the baseline value. In the same fashion the ALT returned within twice the baseline level rapidly but was not completely reduced to baseline until about week 17 or 18 post-challenge (Table IV). Animal 4x36 responded in a similar fashion with peak enzyme values at week 10. In this case GGT levels were more markedly elevated than ALT (Table V). Histologic evidence of infection was totally negative in all three animals following the primary inoculation with heated product. Cytoplasmic tubular structures indicative of NANBV Infection were observed in specimens taken from 4x36 and 4x62 following the unheated challenge dose of NANBV; these changes accompanied the elevation of serum enzymes in the same fashion as that presented by the infected positive control animal (Exhibit A, Tables I, IV, V).

We believe this evidence demonstrates that susceptible animals will not become infected by NANBV of the Hutchinson strain contained in Factor VIII concentrate if the concentrate is processed by the heat treatment tested in this study.

XII. CONCLUSION

None of three chimpanzees receiving an experimental inoculum of heated Factorate^{R} (Armour) demonstrated any sign of infection with NANBV. The product had been seeded with 3000 CHD of NANB (Hutchinson) per vial prior to the

heat treatment. Each chimpanzee received one vial. The infectivity of the unheated product containing the NANBV was verified in a control animal. Of the three chimpanzees receiving the experimental inoculum, two were subsequently challenged with an identical dose of unheated virus to establish the susceptibility of the tested animals to the disease state.

We believe these results conclusively demonstrate the effectiveness of heating a container of lyophilized Antihemophilic Factor at 60°C for 30 hours in destroying the infectivity of a model NANB hepatitis virus up to the tested infectious level of approximately 100 infectious doses per ml of the lyophilized product.

This report finalizes the interim report of the same title which was issued September 20, 1982.

TABLE I

4 x 38 FREDA

POSITIVE CONTROL

EXPERIMENTAL WK	AST	ALT	GGT	E.M.	ANTI HAV	ANTI HBS	HBsAg	ANTI HBC.
-18	18.7	24.8	17.5		-	NA	-	+
-17	9.1	18.6	17.0					
-16	14.8	21.3	15.9					
-15	11.6	24.5	15.4					
-14	8.7	19.4	17.1	NEG.	-	+	NA	NA
-13	7.8	22.8	14.1		NA	NA	-	+
-12	9.2	19.9	16.5					+
-11	9.7	14.9	15.2					
-10	6.6	14.7	15.4					
-09	7.6	18.9	15.7					
-08	11.1	16.8	12.6					
-07	9.2	20.9	14.1					
-06	8.9	18.8	14.7					
-05	9.7	16.2	13.5					
-04	9.9	16.9	13.1					
-03	9.1	17.2	12.3		-	+	-	+
-02	11.0	16.7	13.1					
-01	9.2	18.4	11.7					
inoculation	8.3	18.1	12.7		-	+	-	+
+01	6.9	20.1	12.3	NEG.				
02	11.6	25.2	16.7					
03	8.8	22.8	21.3					
04	7.1	24.0	15.9	NEG	-	+	-	+
05	13.1	29.5	25.0					
06	17.0	33.9	33.4					
07	11.3	45.2	49.3					
08	18.5	57.0	51.7	++	-	+	-	+
09	21.3	87.2	121.2					
10	18.8	81.3	159.7					
11	43.5	121.3	188.1					
12	49.6	193.2	320.8	++	-	+	-	+
13	12.2	87.5	344.8					
14	10.6	81.3	297.1					
15	10.7	40.2	208.7					
16	9.7	25.6	138.8	+	-	+	-	+
17	8.9	23	102.6					
18	9.9	21.6	78.4					
19	7.3	35.2	65.2					
20	7.9	21.2	43.9	NEG				
21	5.5	17.2	44.1					
22	9.5	19.8	31.5					
23	5.2	17.4	26.8					
24	8.1	16.5	17.0	NEG				

AP000281

TABLE II

4 - 58 PETE

POSITIVE CONTROL & CHALLENGE

EXPERIMENTAL WK	AST	ALT	GGT	E.M.	ANTI HAV	ANTI HBS	HBsAg	ANTI HBC.
-5	14.7	15.6	14.1		-	+	-	-
-4	13.0	16.1	13.2					
-3	18.1	23.2	12.8					
-2	15.5	25.0	12.5					
-1	13.4	16.3	12.6	NEG				
inoculation (1)	18.9	17.8	14.3	NEG	-	+	-	+
1	18.9	18.8	11.1					
2	18.2	31.6	15.4					
3	15.2	27.5	18.5					
4	14.2	19.6	18.7	NEG	-	+	-	+
5	14.0	19.3	16.4					
6	13.4	20.0	13.8					
7	11.6	17.0	14.0					
8	15.4	16.6	12.8	NEG	NA	+	-	+
9	14.1	18.0	14.0					
10	14.6	18.5	15.2					
11	13.4	16.0	12.4					
12	13.8	17.0	12.8	NEG	-	+	-	+
13	22.3	20.4	13.4					
14	12.9	17.7	13.0					
15	14.5	18.3	12.9					
16	15.1	16.6	12.5	NEG	-	+	-	+
17	13.9	16.3	12.5					
18	17.2	17.4	11.7					
19	10.0	10.0	10.9					
20	11.6	17.3	10.0					
21	13.9	17.1	13.3					
22	10.4	12.1	12.5					
inoculation (2)	10.1	11.6	13.8	NEG	-	+	-	+
24	22.8	15.1	9.5					
25	16.7	12.8	14.5					
26	13.4	14.1	12.0					
27	12.7	12.0	10.7	NEG	-	+	-	+
28	14.0	15.4	11.6					
29	14.4	16.0	12.3					
30	-	-	-					
31	31.7	18.5	12.2	NEG	-	+	-	+
32	17.6	14.5	12.4					
33	25.8	15.4	9.5					
34	12.7	15.2	11.1					
35	-	-	-					
36	12.6	12.8	11.8	NEG	-	+	-	+
37	16.4	14.7	9.4					
38	15.6	13.5	7.7					
39	10.6	15.2	5.9	NEG	-	+	-	+
40	14.9	13.4	6.7					
41	9.5	10.8	10.9					
42	15.6	14.1	10.2					
43	9.6	11.4	12.6	NEG				
44	11.3	12.0	5.3					

TABLE III

4 x 50 ELAINE

EXPERIMENTAL

EXPERIMENTAL WK	AST	ALT	GGT	E.M.	ANTI HAV	ANTI HBS	HBsAg	ANTI HBC.
-18	13.3	23.5	14.8		-	NA	-	+
-17	9.2	17.1	14.5					
-16	10.2	14.2	14.5					
-15	12.8	17.9	13.5					
-14	11.9	18.8	13.1	NEG	-	+	NA	NA
-13	10.1	20.5	15.3		NA	+	-	+
-12	9.5	23.2	15.7					
-11	12.2	19.7	13.8					
-10	10.6	18.8	14.4					
-09	10.0	22.9	17.3					
-08	10.3	18.2	15.6					
-07	12.8	21.0	16.3					
-06	13.5	21.1	14.7					
-05	11.7	18.1	14.7					
-04	11.3	15.3	15.0					
-03	11.5	18.0	15.9		-	+	-	+
-02	11.0	16.2	16.0					
-01	11.1	20.2	14.7					
inoculation	13.0	18.4	13.6		-	+	-	+
01	10.1	21.8	14.8	NEG				
02	10.9	19.2	13.6					
03	9.7	20.4	14.5					
04	8.1	18.7	20.0	NEG	-	+	-	+
05	10.1	15.3	15.7					
06	11.4	15.8	18.2					
07	10.6	19.7	17.7					
08	11.1	16.2	11.9	NEG	-	+	-	+
09	12.2	21.8	14.8					
10	10.0	15.8	13.0					
11	20.0	22.1	11.2					
12	-	-	-	NEG	-	+	-	+
13	14.5	17.2	14.1					
14	8.4	18.1	13.4					
15	11.8	15.6	14.2					
16	7.2	13.7	14.7	NEG	-	+	-	+
17	8.1	13.2	11.3					
18	8.7	14.3	9.0					
19	9.0	13.9	11.4					
20	7.8	14.8	7.3	NEG				
21	8.4	16.1	6.6					
22	5.8	12.9	13.6					
23	9.5	12.1	10.7					
24	7.4	12.9	13.0	NEG				
25	7.5	11.5	7.4					
26	9.9	12.0	12.8					

TABLE IV

4 x 62 BERT

EXPERIMENTAL & CHALLENGE

EXPERIMENTAL WK	AST	ALT	GGT	E.M.	ANTI HAV	ANTI HBS	HBsAg	ANTI HBC.
-5	16.0	35.4	29.2		-	+	-	-
-4	15.3	27.6	23.3					
-3	14.7	23.7	19.3					
-2	11.4	22.9	17.0					
-1	12.2	27.7	15.6	NEG.	-	+	-	+
inoculation (1)	8.5	26.6	17.1	NEG.	-	+	-	-
1	8.2	28.7	18.4					
2	13.1	28.5	18.3					
3	8.0	24.0	18.4					
4	11.7	30.3	17.8	NEG.	-	+	-	-
5	9.9	29.5	19.5					
6	11.0	27.1	20.9					
7	7.4	25.8	20.5					
8	10.1	23.8	18.0	NEG.	-	+	-	-
9	9.9	29.1	19.2					
10	8.6	26.2	19.5					
11	8.5	28.3	20.4					
12	11.5	26.2	19.9	NEG.	-	+	-	-
13	9.0	27.8	11.9					
14	11.1	24.8	18.6					
15	10.9	24.9	17.7					
16	11.9	29.9	22.1	NEG.	-	+	-	-
17	8.7	28.4	19.8					
18	12.3	35.6	21.2					
19	10.4	27.8	21.9	NEG.				
20	10.4	26.9	20.3		-	+	-	-
21	9.5	30.4	20.2					
22	10.5	29.8	19.6					
inoculation (2)	10.8	27.1	19.5	NEG.	-	+	-	-
24	11.2	29.4	13.9					
25	10.8	27.6	20.5					
26	10.0	31.0	21.7					
27	13.9	31.0	18.5	NEG.	-	+	-	-
28	16.4	45.2	26.2					
29	11.8	47.6	32.2					
30	48.1	153.1	61.5					
31	-	-	-	+	-	+	-	-
32	14.9	71.7	61.1					
33	12.7	51.9	51.0					
34	10.5	48.6	44.4					
35	11.9	40.7	36.5	NEG.	-	+	-	-
36	9.2	36.6	31.6					
37	13.1	38.4	26.5					
38	11.6	31.6	24.8					
39	9.3	33.0	23.3	NEG.				
40	8.3	26.8	17.2					
41	9.1	27.1	22.5					
42	15.4	33.6	11.5					
43	10.4	47.7	24.7	NEG.				
44	9.9	33.6	17.1					

TABLE V

4 x 36 LISA

EXPERIMENTAL & CHALLENGE

EXPERIMENTAL WK	AST	ALT	GGT	E.M.	ANTI HAV	ANTI HBS	HBsAg	ANTI HBC.
-6	11.	16	NA		+	+	-	-
-5								
-4								
-3	10.6	10.5	-		+	NA	-	-
-2								
-1	9.5	11.8	12.5	NEG.	+	+	-	+
inoculation (1)	8.3	11.7	15.7	NEG.	+	+	-	-
1	11.1	15.2	14.9					
2	8.1	14.6	18.8					
3	9.5	14.6	17.6					
4	11.1	19.4	17.6	NEG.	+	+	NA	NA
5	10.3	20.2	19.3					
6	13.9	19.8	19.8					
7	11.9	26.9	17.9					
8	9.3	16.6	17.2	NEG.	NA	+	-	-
9	11.0	17.8	19.5					
10	8.6	18.4	19.2					
11	7.9	13.9	19.0					
12	11.0	16.5	18.5	NEG.	+	+	-	-
13	11.5	16.1	18.2					
14	9.3	14.6	18.0					
15	9.0	15.2	17.0					
16	9.7	13.8	18.4	NEG.	+	+	-	-
17	9.3	15.6	21.0					
18	11.5	17.8	21.0					
19	8.2	19.1	19.9	NEG.				
20	10.1	15.6	18.3		+	+	-	-
21	12.5	17.8	18.8					
22	8.7	14.4	20.5					
inoculation (2)	9.0	16.3	20.5	NEG.	+	+	-	-
24	9.9	16.1	15.4					
25	12.6	19.4	27.5					
26	9.3	21.5	27.7					
27	11.1	28	26.5	+	+	+	-	-
28	11.9	27.9	30.0					
29	14.5	23.5	34.9					
30	12.0	24.8	37.4					
31	-	-	-	+	+	+	-	-
32	13.9	30.2	43.5					
33	25.1	43.6	59.4					
34	11.8	90.3	116.4					
35	9.8	40.4	102.4		+	+	-	-
36	8.1	20.3	77.3	NEG.				
37	11.5	21.4	58.0					
38	11.8	17.6	46.4					
39	6.5	17.1	33.6	NEG.				
40	6.6	16.7	29.3					
41	7.4	14.7	31.8					
42	13.0	18.7	29.6					
43	8.0	14.5	24.3	NEG.				

AP000285

EXHIBIT A

REPORT OF ELECTRON MICROSCOPIC
EXAMINATION OF LIVER BIOPSY SPECIMENS

Southwest Foundation
for Research and Education

West Loop 410 at Military Drive
P.O. Box 28147
San Antonio, Texas 78284
GRO-C

MICROBIOLOGY DEPARTMENT

WORLD HEALTH ORGANIZATION
COLLABORATING CENTER
FOR REFERENCE AND RESEARCH
IN SIMIAN VIRUSES

April 18, 1983

Dr. Michael E. Hrinda
Revlon Health Care Group
1 Scarsdale Road
Tuckahoe, New York 10707

Dear Dr. Hrinda:

Results of electron microscopic examination of the Revlon Non-A/Non-B viral hepatitis study can be seen in the following table. Animals 4-58 and 4X50 were consistently negative for "viral-associated" cytoplasmic tubular structures, whereas liver biopsies from 4X36, 4X38, and 4X62 were found, on occasion, to possess them. These cytoplasmic structures were noted in hepatocytes and were composed of tubular forms (which were circular in cross section) analogous to those found by Shimizu et al, 1979. The membranes of these cytoplasmic tubules were often found in spirals and some were contiguous with endoplasmic reticulum. There was an obvious gradation in terms of number of cytoplasmic tubules found in these specimens. A single plus represents a few cytoplasmic tubules found in hepatocytes, whereas a double plus refers to numerous tubules found, generally speaking, in clusters within hepatocytes. Not all cells possessed these cytoplasmic tubules. The double walled tubular structures were frequently noted and the following electron micrograph depicts the tubules observed in a 4X38 hepatocyte.

Nucleii of these cytoplasmic tubule containing cells were generally normal. Nuclear changes (intranuclear aggregated particles) like those reported by Shimizu et al, 1979, were infrequent and may be of doubtful viral association. Cytoplasmic crystalline arrays were noted in 4X62 (10-27-82) and were similar to those reported by Bradly et al, 1980.

I trust that the enclosed data will satisfy your needs and provide enough detail to complete your study. If I can be of further service to you, please do not hesitate to ask.

Sincerely yours,

GRO-C

G. Con Smith, III

GCS:jb

cc: Dr. J. W. Eichberg

AP000287

Electron Microscopy of Revlon Study
(Non-A Non-B Hepatitis Virus)

Specimen #	Date	4X36	4-58	4X62	4X38	4X50
2693	3-17-82	-*	-	-		
2695	3-24-82	-	-	-		
2697	4-21-82	-	-	-		
2700	4-28-82				-	-
2705	5-19-82	-	-	-		
2709	6-17-82	-	-	-		
2710	7-15-82	-	-	-		
2711	8-04-82	-	-	-		
	8-11-82				-	-
2714	9-01-82	-	-	-	-	-
2718	9-29-82	+	-	-	++	-
2725	10-27-82	+	-	+	++	-
2733	12-01-82	-	-	-	+	-
2738	12-22-82	-	-	-	-	-
2745	1-19-83	-	-	-	-	-
2756	2-16-83		-			

* - means no cytoplasmic tubules
\+ means cytoplasmic tubules

AP000288

Interim Report

Revlon Health Care R&D
Plasma Fractions Division

PFR-82-067

Issue Date 9/20/82

"Hepatitis B Virus Infectivity Assay of Heat-Treated Anti-Hemophilic Globulin Containing Specific Hepatitis Antibody

"The following summary and interim report is for the purpose of accounting status of the experiment. A final report including an interpretation of the results will require the review and approval of all participating scientists and responsible parties before acceptance as a portion of the permanent record.

Prepared By: GRO-C

M. E. Hrinda, Ph.D.
Director, Protein Biochemistry
Participating Scientist in
Study on Behalf of Sponsor

Reviewed By: GRO-C

R. H. Landaburu, Ph.D.
Director, Division of
Plasma Fractions

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AP000289

ARMOUR001180

ARMO0000130_0040

Purpose of the Experiment:

The purpose of the experiment is to determine the infectivity of Hepatitis B virus contained in Antihemophilic Factor using the chimpanzee assay. The Antihemophilic Factor is prepared in two forms. One which contains a quantity of exogenously added antibody to Hepatitis B surface antigen and one which does not contain this added antibody. The rationale is described in detail in the attached exhibit protocol.

Test Substances:

The detailed description of the preparation of test inocula is defined by a written protocol which is attached (EMP#2/1981). Briefly, this preparation is described as follows:

Vials of commercial antihemophilic factor concentrate, Factorate® Generation II (Armour), were infected by adding $10^{3.5}$ CID_{50} of HBV, strain (ay), BOB-NIAID, to each vial following the written procedure. Each vial of this product is normally reconstituted from a lyophilized "cake" with 30 ml of WFI. Factorate® was pooled as a liquid, 30 ml samples retained, and the virus dispensed in the remainder of the pool to achieve an infectivity level of $10^{3.5}$ CID_{50} per 30 ml of solution. Samples of the virus infected pool were aliquoted to vials similarly to the preparation of the commercial product. To the remaining pool of virus-infected product, a quantity of high titered antibody to HBV surface antigen was added. The amount added was determined as 0.27 IU of HBIG per ml of Factorate® solution. For each 30 ml of the virus infected product, this was 0.5 ml of a 1.0% solution of HBIG. The virus infected product was incubated with this globulin under agitation for 60 minutes at ambient temperature, then asceptically aliquoted (30 ml) into vials, frozen and lyophilized. All samples injected into chimpanzees were previously heated (as the lyophilized sealed vial) at 60°C for 30 hours. Subsequent antibody assay of representative vials showed the recovered titer to be 0.21 IU/ml of Factor VIII solution after reconstitution.

The HBV immune globulin was prepared from high titer hepatitis B immune globulin containing human plasma supplied by Plasma Alliance, Knoxville, Tenn. The HBIG was prepared by cold ethanol fractionation using protocols adapted to the laboratory from standard protocols in use at Armour Pharmaceutical Co. for the manufacture of their BOB licensed Normal Immune Serum Globulin. Lyophilized HBIG was dissolved in normal saline at 10 mg/ml, sterile filtered, filled into aliquots and stored frozen. Samples of the bulk lyophilized powder were retained for further study and standardization.

Design of the Experiment:

The execution of the experiment was dictated by the attached protocol. "Hepatitis B Virus Infectivity Assay of Heat-Treated Anti-Hemophilic Globulin Containing Specific Hepatitis Antibody." This protocol required the inoculation of three chimpanzees with Hepatitis B virus infected vials of Factorate® which did or did not additionally contain antibody to Hepatitis B virus. One chimpanzee is designated positive control and received a vial of Factorate® infected with Hepatitis B. Two chimpanzees received vials of Factorate® containing antibody in addition to the virus.

Inoculation of the three test subjects occurred on 3/18/82 following a baseline observation period. The animals selected during the observation period by the study director were:

	Inoculum		
	HBV	Heated	Antibody
Chimpanzee No CH-355 "Raymar"	yes	yes	no
Chimpanzee No CH-373 "Bruno"	yes	yes	yes
Chimpanzee No CH-375 "Tiger"	yes	yes	yes

Because of an uncertainty in two animals during baseline observation (which were rejected), the study director requested histopathologic readings by light microscopy on all animals before inoculation. Dr. Hans Popper of Mt. Sinai judged the animals free of signs of hepatitis. They were likewise seronegative of signs of hepatitis by tests run under protocol. CH-355 "Raymar" had, however, a non-specific elevation of GGT three months prior to inoculation, but this had subsided prior to inoculation.

The protocol requires weekly testing of SGOT (AST), SGPT (ALT), GGT and HBsAg. Additional serum samples are sent to the study director for confirmatory testing. Liver biopsies (percutaneous punch) are performed if serum enzymes become elevated or AUSRIA becomes positive.

Conditions of the Experiment

All participating scientists were required to accept in advance that the experiment would be conducted in compliance with the standards of Good Laboratory Practices. Two written protocols were prepared: (a) the first described and documented the preparation of experimental Factor VIII samples to be used in the in vivo test, and (b) the second described the rationale, procedures and standards for the conduct of the experimental in vivo test. Variances and addenda to these protocols were exchanged and documented. Demonstration copies are attached to this report.

The location of the animals and the conduct of the experiment are at:

The Laboratory for Experimental Medicine
and Surgery in Primates (LEMSIP)
New York University Medical Center
RR#1, Longmeadow Road
Tuxedo, N.Y. 10987

The care accorded the experimental animals is defined by House SOP and Conformance SOP at that Institution. The participating scientists, responsible parties and contracted services are:

Study Director
Friedrick Deinhardt, M.D.
Max von Pettenkofer - Institut
Munich, Germany

Prof. Deinhardt is responsible for the conduct of the study, its design, and scientific evaluation in behalf of the sponsor.

Participating Scientist
Elizabeth Muchmore, M.D.
LEMSIP

Dr. Muchmore is assigned the responsibility as on-site managing scientist for the experiment. She is responsible for assuring that the standards employed by LEMSIP meet the requirements of the protocol for the general care and health of the animals; and for the sampling and communication of raw data to the study director and to the sponsor.

Participating Scientist
Michael E. Hrinda, Ph.D.
Revlon Health Care Group

Dr. Hrinda is responsible as an agent for the sponsor and the study director to monitor the conduct of the study.

Laboratory Services

Routine assays are performed under protocol by the laboratories under the direction of Dr. Saul Krugman, N.Y. University Medical Center. Additional laboratory assays and services are performed under the direction of the study director.

Histologic Preparations
American Histolabs
Rockville, MD.

Contract Pathologist
Hans Popper, M.D.
Mt. Sinai Medical Center
New York, N.Y.

Dr. Popper is responsible for the examination of all histologic specimens on a blind basis; he provides reports of results and communicates the results to Dr. Muchmore for further transmission as described in the protocol.

Results and Interim Status:

The experiment is not fully completed. We are, however, able to draw conclusions on the interim status. The dose of neutralizing antibody chosen for addition to a vial of Factor VIII containing $10^{3.5}$ CID_{50} of HBV strain (ay) was insufficient to render full protection to the test panel of chimpanzees. This negative result is nonetheless in accord with and further supports the work of Tabor et al.[1]:

Following inoculation on 3/14/82, the animals were observed following protocol. The animal designated as positive infection control (CH-355 "Raymar") became antigenemic 13 weeks post-inoculation and reached a peak elevation of serum ALT at week 18. The extent of the elevation (137 IU/l.) was not large, and there was not extensive prolonged antigemia prior to this, but the changes are consistent with the expected course of disease. Antigenemia ceased at 20 weeks post-inoculation with ALT returning to base-line values.

Observation of the two experimental animals, CH-373 "Bruno" and CH-375 "Tiger", did not indicate a similar course of disease or onset in this time window. Both animals demonstrated a very small (peaks 99 IU/l., 70 IU/l.) rise in serum ALT with the peaks occurring 6 and 7 weeks post inoculation. This early peak of ALT was not observed in CH-355 "Raymar". Levels of the other enzymes seemed anomolous, the most obvious was an accompanying rise of GGT in CH-373 "Bruno". No antigenemia (HBsAg) accompanied these small enzyme changes. An early reading of the histologic specimens taken in the study through these points were therefore undertaken. Dr. Popper read the slides and his report is attached following presentation of the tabular data. He found changes in CH-373 Bruno which were described as a mild hepatitis, but could only infer it might be consistent with NANB. Very small changes in CH-375 "Tiger" could not be concluded as anything but a mild hepatic reaction. The observation of biopsies of CH-355 "Raymar", the positive control were negative. Prof. Deinhardt finds the sera in this interval negative for Delta antigen and antibody. Discussion with Prof. Deinhardt indicates he believes these early changes in CH-373 and CH-375 to be non-specific at this point in time - based on the extent, duration and the nature of Dr. Popper's findings.

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In the 20th week post-inoculation, the point at which antigenemia disappeared and serum ALT was near normal in the positive control animal CH-355 "Raymar", CH-375 "Tiger" became positive for HBsAg. A rise in serum ALT did not begin until week 26 post-inoculation. Data in hand and verbal reports on latest findings indicate CH-373 Bruno is negative for all serologic signs of the disease through week 33.

It is known from titration experiments that the incubation interval between inoculation with HBV and onset of disease - is inversely related to the dose of HBV administered. BOB-NIAID strain (ay) inoculum is well studied and established. Where time of onset or peak is used for evaluation, it is clear that the two experimental animals have a delayed or no response to HBV infection. In one case, CH-375, a 7 weeks delay was found and in the other, CH-373, there is no evidence of infection in the 33rd week post inoculation.

As stated earlier, however, it is clear that the addition of 5 mg of purified antibody, which in the highest assay obtained was equivalent to approximately 8.1 IU, was not sufficient to totally neutralize $10^{3.5}$ CID_{50} of the BOB-NIAID inoculum. The presence of "excess" antibody titer in the inoculum vial has been demonstrated to be 0.21 IU/ml. In comparison, the level of anti-HBsAg used in the experiment of Tabor et al.[1] appears to be about 50 fold higher than that in our experiment and it was sufficient to neutralize the infectivity of $10^{3.5}$ CID_{50}. If this latter infective level, used as a challenge dose in both experiments, is the reasonable level of infectivity expected in products, the level of antibody necessary to confer safety is somewhere between that used by us and that used by them. We are aware from a verbal communication of a pending publication that the Netherlands Transfusion Service believes that 0.44 IU/ml of HBIG will confer Hepatitis B safety to Factor VIII products. The results obtained in the present study are consistent with this. Thus, the results of our experiment and the Netherlands acceptance of the HBIG level

0.44 IU/ml as conferring adequate safety against Hepatitis B infection, the addition of HBIG at a titer of 0.5 IU/ml is justified to confer safety against Hepatitis B infection, by our products.

In a final summary of status and interim conclusion, the results of this experiment support the conclusion of a previous preliminary study in another fashion. Heating lyophilized Factor VIII product at 60°C for 30 hours will not eliminate the infectivity of HBV. Infection at this dose is usually expected in 8 weeks though some individual variation is assumed. Onset of infection in the positive control occurred at 13 weeks with antigenemia, the rise of antigen and enzyme levels was not large, and both antigen and enzyme were restored in the 20th week to baseline. It cannot presently be concluded if this is merely an individual response or whether the HBV has been attenuated somewhat by the heating process, since the experiment was not designed to test this hypothesis.