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H.T. Factorate™
Antihemophilic Factor (Human) Dried, Heat-Treated

H.T. Factorate™ Generation II™
Antihemophilic Factor (Human) Dried, Heat-Treated

# SUMMARY OF PRE-CLINICAL AND CLINICAL DATA

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# I. PREFACE

In response to the need for improved safety in Antihemophilic Factor (AHF) preparations, Armour Pharmaceutical Company has developed a heat treatment procedure that has been incorporated into the manufacturing process of Factorate® and Factorate® Generation II™ (Antihemophilic Factor [Human] U.S.P. [Dried]). This heat treatment is designed to reduce the risk of transmitting hepatitis virus to patients under therapy for hemophilia A, although no procedure has been shown to be totally effective in removing hepatitis infectivity from Antihemophilic Factor (Human). Extensive analysis of H.T. Factorate™ and H.T. Factorate™ Generation II™ (Antihemophilic Factor [Human] Dried, Heat-Treated) has been undertaken, and results show that while H.T. Factorate significantly reduces the risk of non-A, non-B (NANB) hepatitis transmission, the basic therapeutic properties of the original Factorate are maintained. Investigators' findings are outlined in detail in this booklet.

With the introduction of H.T. Factorate and H.T. Factorate Generation II concentrates, Armour Pharmaceutical Company reaffirms its full commitment to the preservation of its leadership in product safety. Although there is no simple solution to the virus transmission problem, our success with H.T. Factorate concentrate in the chimpanzee model brings us one step closer to the goal.

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# II. INTRODUCTION

In the last few years, advances in technology have dramatically improved the quality of hemophilia therapy. The availability of AHF, as well as a better understanding of the hemophilia coagulation defect, has permitted more effective and specific therapy, resulting in better control of bleeding and fewer overwhelming orthopedic problems. The insurance provided by AHF availability has permitted a more relaxed medical attitude toward the coagulation defect, and an opportunity to change from "maintenance care" to more comprehensive care.

# III. MINIMIZING THE POTENTIAL FOR HEPATITIS TRANSMISSION

A major goal in improving therapy of patients with hemophilia A has been to limit the potential for transmission of hepatitis virus; specifically, hepatitis B virus (HBV) and hepatitis NANB virus. Both these viruses are thought to be present in the plasma fractions used to prepare AHF concentrate.

## Efforts toward preventing infectivity

Detectable levels of hepatitis B surface antibody (HBsAb) may be indicative of previous exposure to HBV and may have a protective effect against repeated infections, in particular, "acute" hepatitis disease.[3] The benefits of administering maintenance amounts of HBsAb to patients receiving "high risk" blood products have not been determined, but the presence of HBsAb in a product isolated from blood seems to indicate lack of infectivity.

The absence of NANB infectivity in heat-treated albumin and plasma protein fraction (PPF) indicates the susceptibility of this causative agent to destruction by heat. This susceptibility to destruction by heat has also been demonstrated[4] experimentally by administering inocula testing positive for NANB, but heated as per albumin, to chimpanzees.

## The plasma pool—the AHF source, the infectivity source

The accepted industrial procedure for the isolation of therapeutically useful plasma fractions is the cold alcohol fractionation of Cohn and Oncley. Among the products which have gained wide acceptance in therapeutics, immune serum globulin has special production requirements. The most important requirement is that an adequate mixture of clinically important antibodies is present in every lot produced. The Food and Drug Administration requires that a minimum of 1,000 donors contribute plasma to the final antibody mixture.

The opportunity for contamination with blood-transmitted viruses increases with the number of donor-units included in the plasma pool—this is particularly true for those infective agents for which there is no specific assay, such as hepatitis NANB virus. Even for hepatitis B, the "third generation" type assay is not able to detect marginal concentrations of infective particles which carry the potential of infectivity in man. Routine screening of plasma reduces the risk of hepatitis B transmission—perhaps by as much as 25 to 40%.

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There seems to be an inverse relationship between the hepatitis disease incubation period and the number of hepatitis B surface antigen (HBsAg) infective particles injected; however, detection of HBsAg is an indication of the presence of the virus and not a measure of infectivity—infective particles are only those with the capacity to replicate in the receptive host.

Although plasma infected with HBV contains far greater quantities of HBsAg than infective HBV particles, attempts to eliminate the transmission of HBV by eliminating HBsAg positive plasma have not been successful. Even the most sensitive assay for HBsAg detects the antigen only when it is present in large concentrations. Recent reports prepared by the National Center for Drugs and Biologics, and the National Institute of Allergy and Infectious Diseases, U.S.A., indicate that in most cases, the infectivity titer of the inoculum (plasma collected from the infected donor-patient) is greater than the HBsAg detection titer.[5] The stated difference also varies between inocula, adding another factor of uncertainty.

A concentration of $10^8$ HBsAg particles/ml[6] will give borderline or equivocal detection by radioimmunoassay (RIA). One unit of plasma with this concentration provides $10^6$ particles/ml when diluted with 100 non-infectious plasma units, a concentration that is still infectious. The full impact of these virus particle titers can be appreciated when one considers that about 1% of the donor population are carriers of hepatitis B with measurable levels of HBsAg.

The fractionation of plasma protein by the Cohn procedure is accompanied by a differential distribution of infectivity in the different fractions, Fraction III being that which contains the most HBsAg and Fraction II, the least. Fraction II (Immune globulin G) is considered a safe preparation. Human albumin and PPF are routinely pasteurized, and the process has been found to render the products free of hepatitis infectivity.

Fraction I and cryoprecipitate, which contain AHF, are primarily composed of fibrinogen and fibronectin and are not free of HBV. There is no assay available for the detection of hepatitis NANB and consequently, it is impossible to screen plasma for it. NANB virus may be present in every large plasma pool. This infective entity represents a major potential complication in the treatment of hemophilia.[7]

## Efforts to screen the plasma pool

Efforts to obtain plasma fractions that are "non-infectious" have been concentrated in three different areas:

1. *Testing for viruses*: improving the sensitivity in testing for hepatitis virus markers and developing new assays and markers for the identification of viruses, including NANB virus.

2. *Donor selection*: strategies focusing on donor group selection, the size of the plasma pool, and single donor therapy; these strategies have not been sufficient to guarantee the safety of the subsequent products.

3. *Vaccination*: immunization of donors and recipients with available hepatitis B vaccines. The absence of specific markers for hepatitis NANB has hindered progress toward the development of the corresponding vaccine.

## Efforts to improve the plasma fractionation procedure

Evidence that Cohn Fraction II is "free" of hepatitis virus stimulates the pursuit of other procedures which may yield similar results with AHF fractions. Polyethylene glycol fractionation,[5] polyelectrolyte fractionation,[6-8] or fractionation and heating in the presence of high sugar concentrations have all been attempted.[9]

Procedures which selectively eliminate virus particles from therapeutic fractions or preferentially separate them during plasma fractionation have also been investigated. Those that address the preferential separation of hepatitis virus have relied on either a single or a few infectivity evaluations as indications of success. As long as the infectivity titers in the plasma pool vary, the requirements for separating viruses will probably be different for each lot of plasma.

Other approaches to eliminating infectivity include chemical means such as treatment with beta-propiolactone, physical means such as heat treatment, or neutralization by specific immune blockage.[10] The last two approaches will be discussed in more detail.

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# V. EXPERIMENTAL CONSIDERATIONS

## Specifications for the "ideal" plasma fraction

The ideal procedure for eliminating infectivity in plasma fractions remains to be discovered. Nevertheless, some available products and others under development, do claim to be safe from hepatitis transmission. Seldom has the task of defining the precise specifications for the ideal plasma fractionation procedure been more challenged than by the hepatitis safety experimentation with AHF. The resulting plasma fraction should meet the following specifications:

1. Be effective
2. Be biologically acceptable
   - preserve the properties of all protein components of AHF products
   - preserve the therapeutic performance that characterizes the non-treated product
   - carry no long- or short-term risk for the patient
3. Be cost acceptable
   - provide minimal loss of AHF activity
   - provide minimal loss of other proteins with therapeutic value
   - offer simple execution and reliable validation
4. Be endowed with intrinsic characteristics of reproductibility when applied on a large scale

The ideal procedure for eliminating hepatitis should produce a safe product that is otherwise equal to the potentially infective preparation.

## Hepatitis in the experimental setting

The chimpanzee is the only reliable animal model for detecting and evaluating hepatitis B and hepatitis NANB viruses. For hepatitis experimentation, special care in the handling of these animals is necessary and has been described.[11] Basic conditions for including an animal in hepatitis infectivity studies are:

- absence of exposure to potential sources of HBV
- absence of serologic markers for HBV
- absence of any liver deficiency whether or not the causes of the deficiency are known

When evaluating NANB infectivity, the requirements for the "quality" of the experimental animal are more lenient. Young animals (those that are colony born from healthy breeders and have not been exposed to potential sources of infection), are preferred.

The inoculum that is used should be one that is well-documented, kept under conditions which guarantee its preservation and one that gives consistent results when administered repeatedly.

## Hepatitis B virus (HBV)

*Subtype adw may be the least reliable inoculum for determining infectivity.*

Three HBV inocula with the following subtypes of HBsAg are available from the National Center for Drugs and Biologics and the National Institute of Allergy and Infectious Diseases: ayw, adr and adw. These inocula have been extensively evaluated in chimpanzees. One ml was found to have an infective potency of $10^{7.5}$ Chimpanzee Infective Dose ($CID_{50}$) (ayw), $10^{8.0}$ $CID_{50}$ (adr), and $10^{7.0}$ $CID_{50}$ (adw). The infectivity as defined by the length of the incubation period is inversely related to the number of infectious particles inoculated. Subtype adw may be the least reliable inoculum, especially at low infective doses (dilution of over $10^{3}$), but caution is required when interpreting prolonged incubation periods as a reflection of diminished infectivity.[5] The incubation period in NANB infection is apparently less consistent than for HBV infection.

## Hepatitis non-A, non-B (NANB) virus

*In animal studies of NANB infectivity, no definitive conclusions are justified unless the negative results obtained are rechallenged.*

The inocula identifiable for NANB infectivity usually have low infectivity titers. An exception is the Hutchinson pool, which has consistently produced elevated liver enzyme levels and characteristic cytoplasmic and nuclear changes at dilutions of $10^{-6}$. In chimpanzees, dilutions from $10^{-2}$ to $10^{-6}$ produce initial alanine aminotransferase (ALT) elevations at three and five weeks, with peak elevations occurring at approximately seven weeks post-inoculation.[12]

Since an undetermined proportion of infectivity may be inapparent in the host, the NANB infectivity is expressed in Chimpanzee Hepatitis Doses ($CHD_{50}$). Histologic changes characteristic of NANB infection may be observed without diagnostic liver enzyme elevations. The reverse is also true. It is therefore imperative that infectivity studies for NANB virus be followed by rechallenge of all negative results through the administration of a known infectious formulation of the inocula. This is especially important in studies with a small number of animals; the receptivity of the animal model and the infectivity of the inocula must be controlled. No definitive conclusions are justified unless negative results are rechallenged.

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## The procedure for biochemical evaluation

Detailed instructions for evaluating animals pre- and post-inoculation vary from laboratory to laboratory. The biochemical evaluations in our studies included the following assays:

*Pre-inoculation*: Serum Glutamic Pyruvic-Transaminase (SGPT), Serum Glutamic Oxalacetic-Transaminase (SGOT), Gamma Glutamyl-Transpeptidase, Isocitric Dihydrogenase (ICD) or Serum Alanine Aminotransferase (ALT) x 6; HBsAg (AUSRIA), Anti-HBs (AUSAB), Anti-HBc (CORAB), Anti-HAV (HAVAB) x 2; liver biopsy x 2 (light microscopy, electron microscopy and immuno-fluorescence microscopy).

*Post-inoculation*: Weekly serum, SGPT, SGOT, ICD or ALT. Monthly AUSRIA, AUSAB, CORAB, HAVAB. Liver biopsy once monthly, once weekly if enzymes are elevated.

*The Armour heat-treatment procedure has been tested in chimpanzees under conditions which were extremely rigorous. The HBV subtype used was considered to be the most reliable, and doses of hepatitis B virus and NANB virus were both set purposely high—at 3,000 $CID_{50}$ and 3,000 $CHD_{50}$, respectively.*

## Minimizing infectivity through the use of heat

The plasma fractionation industry has made extensive use of heat in the pasteurization of albumin and albumin-containing products. Although heat has been applied before in the preparation of AHF, the procedure has resulted in costly losses in protein activity.[9] Apparently, there is a minimum temperature level at which HBV is sensitive and a maximum temperature level the desirable properties of the product can withstand. Heat exposure-time is the most important factor. Heat should be applied for as long as it takes to destroy the virus, but not so long that the characteristics of the basic product are compromised. The parameters of heat application have to be established through experimentation, and should be tested under the most rigorous experimental design.

Our heat treatment has been tested in chimpanzees at 3,000 $CID_{50}$ of hepatitis B virus per chimpanzee, an infective dose so high that it would not be likely to go undetected in a product purified from HBV-screened plasma. The HBV subtype ayw we used was also documented as being the type most likely to give reliable responses in chimpanzees.[5]

The Hutchinson pool of NANB virus, known to have an infectivity titer of at least $10^6$, was used to challenge our initial results. An infectivity dose higher than levels likely to be found in the plasma of infected patients, 3,000 $CHD_{50}$, was also a dose likely to exceed levels of infectivity in products purified from such plasma.

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# V. EXPERIMENTAL STUDIES

## Heat in HBV infectivity

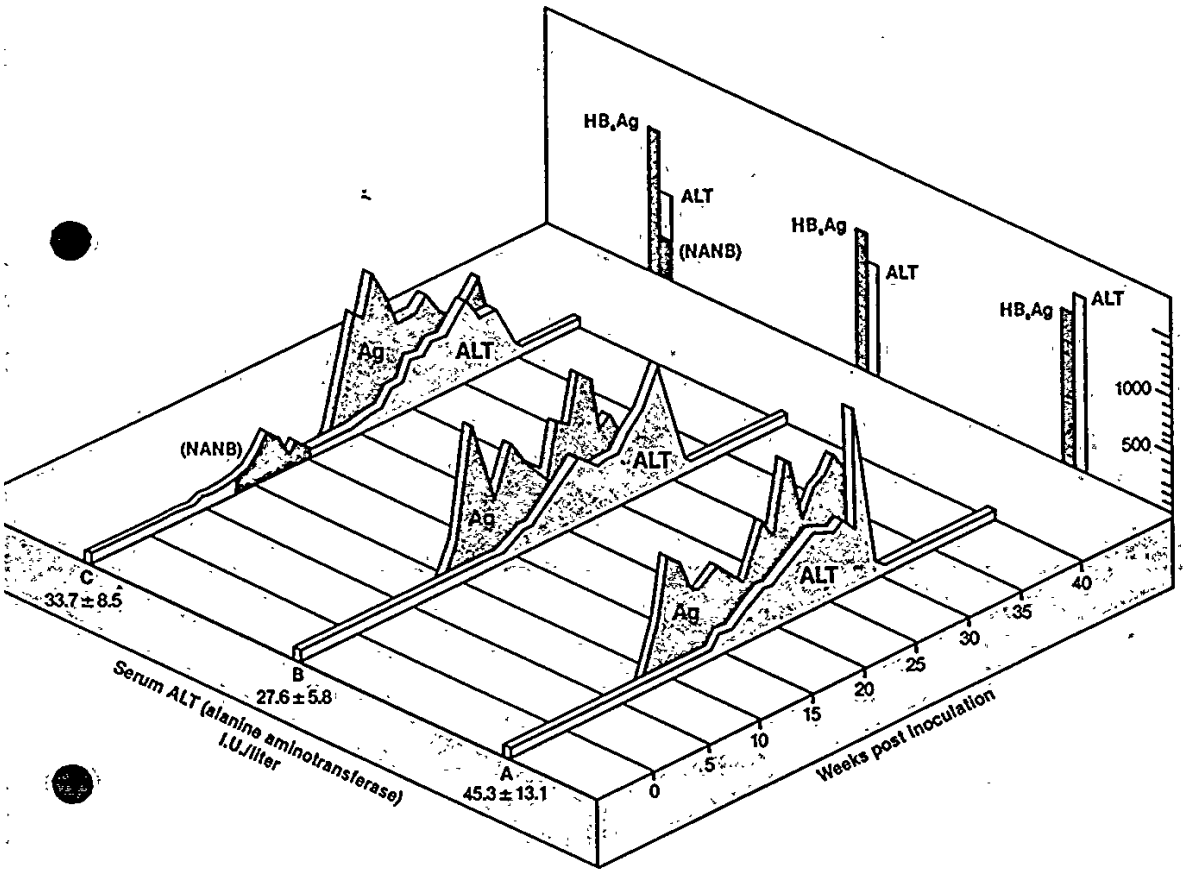
The purpose of our study was to determine if a measured quantity of HBV added to the Armour Antihemophilic Factor preparation could be destroyed by heating the AHF preparation under standard conditions. The results clearly indicated that AHF, seeded with approximately 3,000 infectious hepatitis B viruses per inoculum and then heated, was still highly infectious for chimpanzees. There was no evidence of attenuation or partial inactivation as judged by the length of the incubation period from inoculation to appearance of HBsAg. (See Figure 1, A and B.)

Of more interest was the observation that a control animal receiving the unheated preparation developed hepatitis NANB with the characteristic cytoplasmic abnormalities detectable by light microscopy. (See Figure 1, C.) Evidence of hepatitis NANB was followed by evidence of HBV infection. *Thus, the animal had two sequential infections with hepatitis*; the first infection was unpredictable and presumably produced by NANB viruses present in the AHF preparation, and the second infection was produced by the known HBV seeded in the inoculum. Animals receiving the heated preparation did not develop NANB hepatitis.

There is more than one explanation for the above results; one interpretation, however, is that hepatitis NANB virus was inactivated by heat, but not HBV. Another explanation was that the infective NANB agent in the AHF was not present at a high enough concentration to produce consistent infectivity. Most evaluated plasma from hepatitis NANB donors is found to have a low infectivity titer, ranging between $10^1$ and $10^3$.

Figure 1.

Hepatitis infectivity assay (Hepatitis B inoculum, MS-2 POOL (ayw) 3,000 $CID_{50}$; Hepatitis NANB, unknown titer in study product)




A + B = HBsAg and serum ALT levels after inoculation of heated AHF
C = HBsAg and serum ALT after inoculation of unheated AHF

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## Heat in NANB infectivity

It has been suggested that a minimum of $10^3$ Chimpanzee Hepatitis Doses ($CHD_{50}$) is required for NANB virus experimentation with chimpanzees. Initial experiments did not fulfill this basic experimental requirement, so the data were not conclusive for clear interpretation. These observations, needing re-confirmation, were tested by repeating the experiment with an appropriately titrated infective dose of NANB virus. The Hutchinson pool, known to have an infectivity titer of at least $10^6$, was used for the challenge.

The purpose was to confirm that an infective measured quantity of a documented NANB virus could be destroyed through the application of heat. A total of 3,000 $CHD_{50}$ were injected in each one of the experimental animals in AHF vials simulating those that are commercially manufactured as Factorate® (Antihemophilic Factor [Human] U.S.P. [Dried]). Identical vials were reserved for rechallenge.

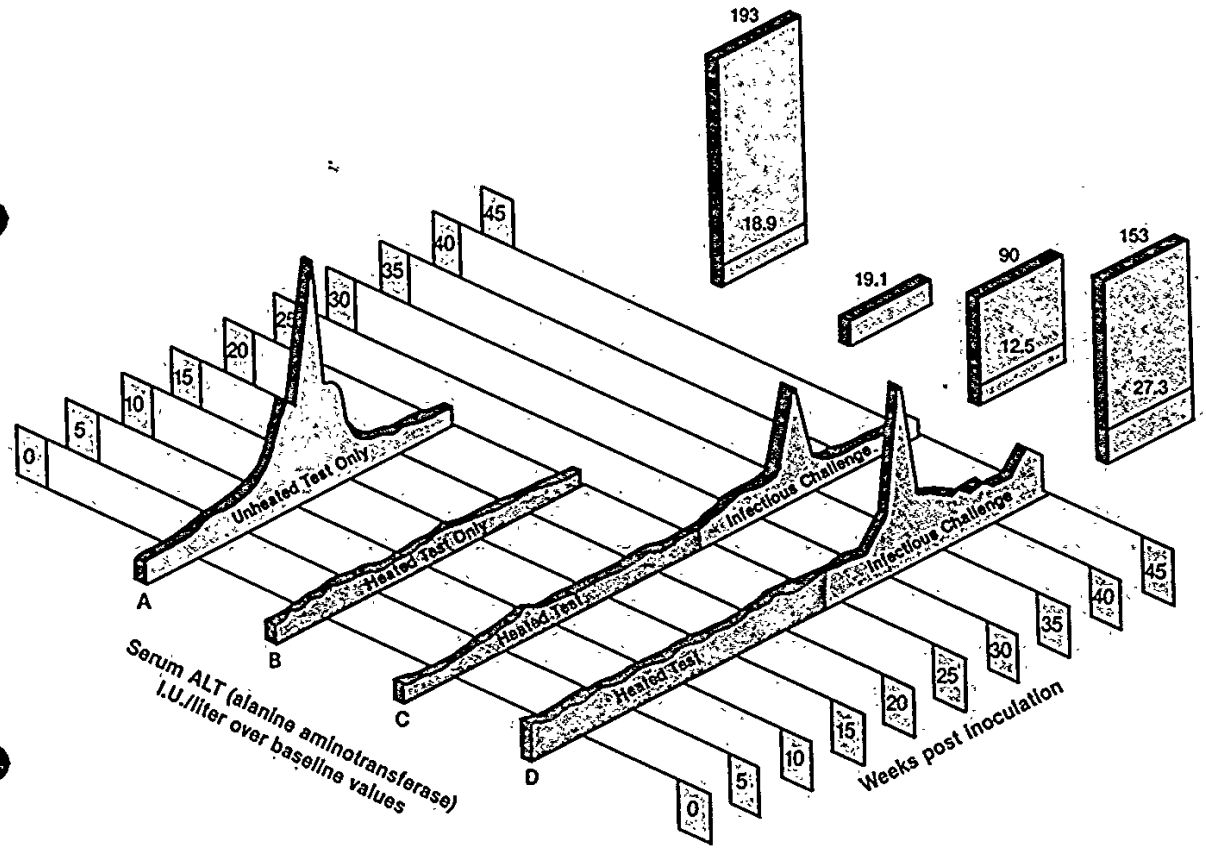
*The purpose of our study was to confirm that an infective measured quantity of a documented NANB virus could be destroyed through the application of heat.*

In this experiment, the heating process was successful in eliminating NANB infectivity from the AHF product tested. The positive control animal that received unheated AHF and NANB virus developed enzyme elevation within the expected incubation period. (See Figure 2, animal A.) The three experimental animals that received heated AHF and NANB virus remained normal and enzyme activity was maintained at baseline values. (See Figure 2, animals B, C & D.) Changes in liver structure were only evident in the positive control animal (animal A).

The results of the experiment were further validated by reinjecting two of the NANB, negative chimpanzees (animals C & D), with inoculum identical to that received by the same animals at the initiation of the experiment. However, this time, the inoculum was not heated. NANB hepatitis developed within the expected incubation time, indicating that the initial results were not due to lack of inocula infectivity or to animal resistance to infection. These findings confirmed the potential of the Armour heating process to minimize NANB infectivity in AHF products.

Figure 2.

Non-A, non-B hepatitis infectivity assay (Inoculum—Hutchinson strain 3,000 $CHD_{50}$ per injection)




A = ALT levels subsequent to inoculation of unheated AHF
B = ALT levels subsequent to inoculation of heated AHF
C + D = ALT levels subsequent to inoculation of heated AHF followed by rechallenge with unheated AHF

*See prescribing information on last pages.*

# II. PRODUCT CHARACTERIZATION

## The effects of heat treatment

Factorate® (Antihemophilic Factor [Human] U.S.P. [Dried]), a protein concentrate derived from human plasma, and containing at least 20 times the concentration of AHF (Factor VIII:C) present in normal plasma, was biochemically evaluated after heat treatment.

The experimental protocol was also designed to *evaluate the effect of the heat treatment on the proteins present in Factorate*, e.g., fibrinogen, fibronectin, gamma globulin and albumin as well as trace amounts of other plasma proteins. Heat treatment that would reduce the concentrate's potential infectivity without altering its biochemical or biological properties would significantly improve the overall value of the preparation in hemophilia therapy.

Long-term evaluations of the stability of Factor VIII:C, along with evaluations of the components of the product were used in ascertaining the effects of heat on all proteins present in the concentrate. Particular emphasis was given to the immunological detection of neo-antigens. These can serve as a sensitive indicator of protein structure alteration, which in turn, can lead to eventual sensitization or resistance to specific therapy.

## Description of test products

Factorate® or Factorate® Generation II™ (Antihemophilic Factor [Human] U.S.P. [Dried]) stored between 2°C–8°C were used as controls in the studies. The experimental concentrates were heat-treated and then refrigerated. At selected time intervals, heat-treated Factorate and a control were reconstituted with water for injection (as described in the Factorate package insert), and analyzed.

## Experimental protocol

In addition to evaluations of the long-term stability of Factor VIII:C, immunological methods were used to detect neo-antigens; spectrophotometric and electrophoretic analyses were employed to ascertain changes in protein structure, and heated concentrate was evaluated for retention of specific protein identity. The treated product was also evaluated for conformity with the specifications for licensed Factorate. Methods of analyses were conducted as follows:

Factor VIII:C–Factor VIII:C coagulant activity was determined by one- and two-stage assays. These data were analyzed using a statistical program for validation with a 95% confidence rate. An Armour Pharmaceutical Company reference material previously standardized against the Second International Factor VIII Standard (Human) 73/552 (WHO No. 2) was used as the reference standard.

**Electrophoretic migration**–Electrophoretic migration on polyacrylamide gel was used to compare protein patterns in the samples. The distribution of the proteins was compared through computer analysis of densitometric scans of the stained gels.

**Solubility**–Solubility was determined by reconstitution time.

**Moisture content**–A Photo Volt Aquatest IV apparatus was used to determine the moisture content by the Karl Fischer method.

**Spectral analysis**–The visible or fluorescent spectra of heat-treated Factorate were studied since the detection of new peaks, or a shift in the spectra, would be indicative of a change in the native structure of the proteins. The visible spectrum of the heat-treated and control samples was compared using a spectrophotometer.

**Thrombin Activation of Factor VIII:C**–Factor VIII:C coagulant activity has been shown to be enhanced by incubation with trace amounts of thrombin. The marked increase in activity is usually of short duration and cannot be induced if the factor has been previously exposed to thrombin. Purified human thrombin was used to compare coagulant activity in the treated and unheated products. Samples were incubated for up to 30 minutes with 5 ng of human thrombin and assayed by one-stage PTT at different incubation times.

**Neo-Antigens**–Immunological methods were used to detect the presence or absence of neo-antigens. Rabbits were injected with heat-treated or unheated Factorate in a complete Freund's adjuvant over a period of six weeks, and bled periodically for three months. Heat-treated and control Factorate samples were analyzed by two-dimensional electrophoresis using the corresponding rabbit antisera.

Electrophoresis of 5 microliters of sample in the first dimension was run at 10°C at 10V/cm for one hour. Electrophoresis in the second dimension was run in an agarose gel containing 0.25 ml of antiserum with antibodies against heat-treated or control Factorate per plate.

**Gel diffusion**–Ouchterlony gel diffusion was used to compare the antigenic determinants of IgG, albumin, $\alpha$-fibrinogen, $\beta$-lipoprotein, $\gamma$-lipoprotein and transferrin in heat-treated and control samples using monospecific antibodies. If any of these proteins were altered by the heat treatment, modifications in protein structure would be detectable as a loss of identity with the same proteins contained in the untreated samples.

*See prescribing information on last pages.*

**Immunoglobulin content**–The IgG, IgA and IgM content of Factorate® (Antihemophilic Factor [Human] U.S.P. [Dried]) was determined by using Behring Partigen Plates and by measuring radial immuno-diffusion.

* * *

The results of these studies demonstrated that the distinguishing properties of Factorate concentrate were retained in the heat-treated preparation:

- No significant stability differences were noted between lots of heat-treated and control Factorate concentrate assayed for Factor VIII:C activity over a period of one year. (See Table 1.)

TABLE 1

Comparison of Factor VIII:C (units/ml) in Factorate and H.T. Factorate™ (Antihemophilic Factor [Human] Dried, Heat-Treated)

| Sample | Initial Potency (units/ml) | Months | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 6 | 12 |
| **Lot 1** | | | | | | |
| Factorate | 24.2 | 24.0 | 23.8 | 23.6 | 22.9 | 21.6 |
| H.T. Factorate | 22.5 | 23.5 | 23.1 | 23.4 | 22.7 | 21.0 |
| **Lot 2** | | | | | | |
| Factorate | 27.5 | 27.4 | 27.2 | 27.1 | 26.8 | 25.9 |
| H.T. Factorate | 26.1 | 26.3 | 26.3 | 25.8 | 25.7 | 24.9 |
| **Lot 3** | | | | | | |
| Factorate | 26.1 | 25.8 | 25.7 | 25.6 | 25.3 | 24.5 |
| H.T. Factorate | 23.0 | 24.0 | 24.0 | 23.5 | 23.0 | 22.3 |

- The electrophoretic migration from the origin of the heat-treated Factorate concentrate proteins was the same as in the untreated sample. (See Table 2.)
- Visible and fluorescent spectra of heated Factorate concentrate was indistinguishable from that of the untreated sample. No peak shifts or new peaks were detected.

TABLE 2

Comparison of RF values* of the major protein constituents of Armour Antihemophilic Factor (Human) preparation, heat-treated and unheated

| | Heat-treated | Unheated |
|---|---|---|
| Fibronectin | 0.120 ± 0.002 | 0.125 ± 0.004 |
| Fibrinogen | $\alpha$ 0.516 ± 0.008 | 0.514 ± 0.011 |
| | $\beta$ 0.624 ± 0.001 | 0.617 ± 0.007 |
| | $\gamma$ 0.658 ± 0.008 | 0.654 ± 0.008 |
| Albumin | 0.662 ± 0.008 | 0.670 ± 0.017 |

*Means ± S.D.

- The reconstitution time of heat-treated samples remained unchanged, and the solution clear and colorless.
- The moisture content of the test vials containing heat-treated or unheated Factorate concentrate remained consistent from lot to lot.
- The Factor VIII:C in unheated and heat-treated product showed the same 10- to 15-fold increase in activity after two-to-three minutes of incubation with thrombin. (See Table 3.)
- In rabbits, no exposure of new antigenic sites was demonstrated. Identical, two-dimensional patterns were obtained when heat-treated and unheated Factorate concentrate were electrophoresed in antisera-containing plates.

*See prescribing information on last pages.*

TABLE 3

Maximum response of unheated and heat-treated Factorate®
(Antihemophilic Factor [Human] U.S.P. [Dried]) to thrombin*

| Sample | VIII Activity | Thrombin Activated VIII Activity | X-Fold Activity |
|---|---|---|---|
| Unheated Factorate | 7.0 | 99 | 14 |
| Heat-treated Factorate | 7.2 | 75 | 10 |

*Thrombin was added to a level of 5 hg/ml, of the mixture being assayed.

- Lines of identity were found for each of the proteins in the heat-treated and unheated samples. These lines were apparent after aliquots of heat-treated and unheated Factorate concentrate were applied to ouchterlony plates containing specific antibodies against IgG, albumin, $\alpha$-fibrinogen, $\beta$-lipoprotein, $\gamma$-lipoprotein and transferrin. The plates were examined after 24, 48, and 72 hours and there was no evidence of spur formation.
- The immunoglobulin content of heated Factorate concentrate remained unchanged when analyzed by immunological methods and represents a small percent of the protein content of the sample.

The information provided by these *in vitro* studies indicates that the biological, biochemical and immunological properties of the proteins in Factorate concentrate are retained in their native state in the heat-treated product. The heat-treated form of Factorate concentrate conforms with the accepted criteria for a safe, nonpyrogenic, sterile product for therapeutic use.

# VII. ANIMAL SAFETY AND EFFICACY STUDIES

## Toxicology studies

Factorate concentrate has been tested extensively in several animal species by a number of routes of administration without evidence of toxic effect. H.T. Factorate™ (Antihemophilic Factor [Human] Dried, Heat-Treated) has undergone the same testing with similarly favorable results.

## Safety study in normal dogs

Normal dogs were infused with unheated or heat-treated product at the rate of 4.4 ml/min over a period of 4.5 minutes at a dose of 100 units/kg. Heart rate and mean arterial pressure were monitored during the study.

Infusions of unheated or heat-treated Factorate concentrate produced equivalent insignificant transient variations in heart rate and mean arterial blood pressure.

## Efficacy studies in dogs with hemophilia A

Dogs with hemophilia A were infused with heat-treated or unheated Factorate concentrate at a rate of 8 ml/min over a four-minute period with a total dose of 770 units of Factor VIII:C. Venous blood was collected periodically over a 24-hour, post-infusion period for Factor VIII:C and Factor VIII related antigen (Factor VIII:RA) analysis, hematocrit, white cell and platelet counts. Respiration rate, pulse pressure and temperature were also monitored.

Plasma levels of Factor VIII:C and Factor VIII:RA were similarly increased after infusion with the heat-treated and unheated samples. (See Table 4.) There was no change in hematocrit, total blood protein or temperature during or after infusions. Although there were fluctuations in white blood cell and platelet counts, these were not considered abnormal.

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TABLE 4

Plasma levels* of Factor VIII:C and Factor VIII:RA in hemophilic dogs administered heat-treated and unheated Factorate® (Antihemophilic Factor [Human] U.S.P. [Dried])

| Time (hours) | Unheated Factorate | | Heat-Treated Factorate | | | |
|---|---|---|---|---|---|---|
| | | | DOG A | | DOG B | |
| | VIII:C | VIII:RA | VIII:C | VIII:RA | VIII:C | VIII:RA |
| 0 | 0.38 | 1.58 | 0.19 | 0.98 | 0.35 | 1.07 |
| 0.25 | 0.67 | 1.99 | 0.68 | 1.43 | 0.82 | 1.62 |
| 1.0 | 0.63 | 1.82 | 0.63 | 1.43 | 0.68 | 1.74 |
| 1.5 | 0.53 | 1.63 | 0.52 | 1.43 | 0.68 | 1.76 |
| 3.0 | 0.45 | 1.62 | 0.43 | 1.42 | 0.64 | 1.66 |
| 5.0 | 0.43 | 1.70 | 0.38 | 1.45 | 0.56 | 1.69 |
| 8.0 | 0.43 | 1.35 | 0.32 | 1.36 | 0.55 | 1.55 |
| 24.0 | 0.33 | 1.17 | 0.27 | 1.01 | 0.47 | 1.33 |

* Expressed in units/ml

# VIII. SAFETY AND EFFICACY STUDIES IN HEMOPHILIACS

A two-way crossover study comparing the safety and efficacy of the Armour heat-treated Antihemophilic Factor, H.T. Factorate™ (Antihemophilic Factor [Human] Dried, Heat-Treated), and unheated concentrate derived from the same parent lot, Factorate concentrate (Antihemophilic Factor [Human] U.S.P. [Dried]), was conducted in six hemophilia A patients ranging in age from 22 to 50 years. Prior to receiving the concentrates, patients were evaluated by medical history, physical examination, complete blood chemistry, urinalysis, hemogram, coagulation profile and ECG. The patients' vital signs were monitored during and after the studies.

Pre-infusion coagulation profiles of the patients verified that their only clotting deficiency was a low level of Factor VIII:C. (See Table 5.) All patients were found to have measurable levels of hepatitis B antibody, but the corresponding antigen could not be detected. One patient had markedly elevated preinfusion levels of SGOT and SGPT, four patients demonstrated mildly elevated liver enzyme activity, and the sixth patient

TABLE 5

Preinfusion coagulation profiles of hemophilia A patients

| Patient | Age | Factor VIII:C level (%) | Factor VIII:RA level (units/ml) | Ristocetin co-factor activity (units/ml) | Prothrombin time patient/control (seconds) |
|---|---|---|---|---|---|
| 1 | 24 | 1 | 199 | 128 | 11.3/11.2 |
| 2 | 23 | 1 | 118 | 117 | 10.9/11.2 |
| 3 | 22 | 1 | 216 | 78 | 10.4/11.2 |
| 4 | 27 | 2.3 | 117 | 117 | 11.4/11.6 |
| 5 | 50 | 2.7 | 162 | 158 | 11.0/11.6 |
| 6 | 28 | 1 | 170 | 137 | 10.6/11.6 |

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was within the normal range. None of the patients demonstrated an increase in SGOT or SGPT during or after infusion of H.T. Factorate™ (Antihemophilic Factor [Human] Dried, Heat-Treated) or unheated Factorate® (Antihemophilic Factor [Human] U.S.P. [Dried]). Blood chemistry, urinalysis, hematocrit, hemoglobulin, white blood cell, platelet, and red blood cell readings were maintained at preinfusion levels, and remained within normal limits throughout the study.

In general, there were no laboratory abnormalities that could be attributed to the use of Factorate. It appeared that the heated product was free of adverse reactions.

The half-life and recovery of Factor VIII:C after infusion of heat-treated and unheated Factorate concentrate were 10.8 ± 3.9 hours and 99.9%, and 10.9 ± 3.6 hours and 96.3%, respectively. (See Table 6.) The similarity of these results demonstrates that the heat-treated product is therapeutically equivalent to unheated Factorate concentrate.

TABLE 6

The *in vivo* Factor VIII:C half-life and recovery after infusion of Factorate or H.T. Factorate concentrates

| | Factorate | | H.T. Factorate | |
|---|---|---|---|---|
| Patient | Half-life (hrs) | Recovery (%) | Half-life (hrs) | Recovery (%) |
| 1 | 8.5 | 87.3 | 8.5 | 91.7 |
| 2 | 9.4 | 77.3 | 8.0 | 103.3 |
| 3 | 8.3 | 99.0 | 8.4 | 94.0 |
| 4 | 16.0 | 91.3 | 17.0 | 89.7 |
| 5 | 14.9 | 105.3 | 14.2 | 116.0 |
| 6 | 8.4 | 117.3 | 8.5 | 105.0 |
| Mean | 10.9 | 96.3 | 10.8 | 99.9 |
| Standard Deviation | 3.6 | 14.1 | 3.9 | 10.0 |

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