FDA OVERSIGHT: BLOOD SAFETY AND THE IMPLICATIONS OF POOL SIZES IN THE MANUFACTURE OF PLASMA DERIVATIVES

HEARING

BEFORE THE

SUBCOMMITTEE ON HUMAN RESOURCES

OF THE

COMMITTEE ON GOVERNMENT REFORM AND OVERSIGHT

HOUSE OF REPRESENTATIVES

ONE HUNDRED FIFTH CONGRESS

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FDA OVERSIGHT: BLOOD SAFETY AND THE IMPLICATIONS OF POOL SIZES IN THE MANUFACTURE OF PLASMA DERIVATIVES

THURSDAY, JULY 31, 1997

HOUSE OF REPRESENTATIVES,
SUBCOMMITTEE ON HUMAN RESOURCES,
COMMITTEE ON GOVERNMENT REFORM AND OVERSIGHT,
Washington, DC.

The subcommittee met, pursuant to notice, at 10:05 a.m., in room 2247, Rayburn House Office Building, Hon. Christopher Shays (chairman of the subcommittee) presiding.

Present: Representatives Shays, Snowbarger, Pappas, Towns and Kucinich.

Ex officio present: Representative Burton.

Staff present: Lawrence J. Halloran, staff director and counsel; Anne Marie Finley, professional staff member; R. Jared Carpenter, clerk; Cherri Branson, minority counsel; and Ellen Rayner, minority chief clerk.

Mr. SHAYS. I would like to call this hearing to order.

Welcome to our witnesses and our guests. To minimize the risk of injury or death in the event of an emergency, the fire safety laws set a maximum on the number of people allowed in this room.

This was not a good way to open.

Surprisingly, the blood safety laws don't contain the same type of common-sense safeguard. There are currently no limits on the number of blood plasma donations combined into the pools from which therapeutic proteins are extracted or fractionated. In the event of an emergency such as the appearance of a new blood-borne infectious agent, excessively large plasma pools increase the risk of disease transmission to the users of plasma-derived products, and make recalls more difficult.

A user of a single dose of a fractionated product today may be exposed to plasma from as many as 400,000 donors. Pool sizes vary widely from company to company, product to product, lot to lot, dose to dose. There is no standard.

Patients are not routinely informed of the risks associated with plasma pool sizes. Last year, in our oversight report on blood safety, we recommended, among other steps, that plasma fractionators should limit the size of plasma pools, with pool sizes determined as much by public health risk factors as by production economies of scale.

Today, we ask Federal public health agencies, blood product consumers, and the plasma industry what progress has been made

bringing safety considerations to bear and setting practical upper limits on plasma pool sizes. For some products, pooling is beneficial, even required, to capture a broad range of antibodies, for example, or to extract a sufficient volume of a scarce protein. For other products, however, there is an undeniable and direct relationship between the number of donors in the plasma pool and the risk of exposure to an undetected infectious agent.

Tragedy taught us that lesson. In the early 1980's, new hepatitis strains and the human immunodeficiency virus, HIV, slipped into the blood supply. Thousands died. Hundreds of thousands were exposed to Hepatitis C, many of whom have never been told of their possible infection.

Now other viral agents, and perhaps prion diseases, pose similar threats to the safety of the blood supply. Yet the risks presented by pool sizes have not been addressed.

Why? Because some believe pool size limits are unnecessary, even imprudent. Others tell virtually any production pool limits will have long-term negative effects on the availability and costs of needed medical therapies. One recent study concluded pool size reductions offer only marginal added safety for frequent and chronic plasma product users. This study suggests as much or more could be achieved by focusing on other aspects of the blood safety system—donors screening, viral inactivation, more aggressive disease surveillance.

But the vigilance required to maintain a safe blood supply demands we avoid false choices between safety and supply, and pursue every reasonable risk reduction strategy. Given the known vulnerabilities of the donor screening and product recall process, it is not plausible to expect those aspects of the safety system to bear all the burden of excluding or retrieving the infectious agents present in plasma products as a function of pool size. As long as production pool sizes remain the only aspect of the entire process not in any way delimited by some safety considerations, we tolerate avoidable risk. This is intolerable.

Plasma pool size limits could serve as a fire wall against the spread of a new infectious agent, particularly one that is not yet widely distributed or for which no detection or inactivation technology has been developed. However transitional or brief, the added safety margin afforded by practical pool size limits could last some plasma product users a lifetime.

We are fortunate, and grateful, to have witnesses before us today who are expert in every aspect of this issue: public health, clinical usage, safety, efficacy, blood supply, and cost. The subcommittee appreciates their being here today, and we look forward to their testimony.

At this time, I recognize the gentleman from New Jersey.

[The prepared statement of Hon. Christopher Shays follows:]

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Statement of Rep. Christopher Shays
July 31, 1997

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Today we ask federal public health agencies, blood product consumers and the plasma industry what progress has been made in bringing safety considerations to bear in setting practical upper limits on plasma pool sizes.

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We are fortunate, and grateful, to have witnesses before us today who are expert in every aspect of this issue: public health, clinical usage, safety, efficacy, product supply and cost. The Subcommittee appreciates your being here, and we look forward to your testimony.

Mr. PAPPAS. Thank you, Mr. Chairman. I, too, want to thank the panelists for being here. I am equally as interested in this issue. I appreciate the chairman's leadership in calling this matter.

Mr. SHAYS. Thank you.

I ask unanimous consent that all members of the subcommittee be permitted to place any opening statement in the record and that the record remain open for 3 days for that purpose.

And without objection, so ordered.

I ask unanimous consent that all witnesses be permitted to include their written statements in the record, and without objection, so ordered.

And I will just mention that the ranking member wanted us to proceed, but we may interrupt your testimony to allow him to make a statement and to put it in the record.

At this time, the committee calls before us panel one: David Satcher, Director, Centers for Disease Control and Prevention; Paul Brown, senior Research Scientist, Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health; and Kathryn Zoon, Director of Center for Biologics Evaluation and Research, Food and Drug Administration.

At this time, we are going to let our ranking member take his breath, sit down, and make a statement.

Mr. TOWNS. Mr. Chairman, for the first time in all the years you have known me, I would just like to submit my statement for the record and let you move forward with the witnesses.

Mr. SHAYS. OK. Thank you. I acknowledged that you allowed us to start earlier and we thank you because we have a long and very interesting day.

[The prepared statement of Hon. Edolphus Towns follows:]

Congress of the United States

House of Representatives

OPENING STATEMENT

REP. EDOLPHUS TOWNS
RANKING DEMOCRATIC MEMBER

BEFORE THE
SUBCOMMITTEE ON HUMAN RESOURCES

JULY 31, 1997

Mr. Chairman, I want to thank you for holding today's hearing to examine the role of plasma pool size and the potential connection between CJD disease and the safety of the blood supply.

In preparation for this hearing, I was surprised to learn that there are over 500 products which use plasma products or derivatives either in manufacturing or formulation. Clearly, each dose of each product may present a risk unless proper precautions have been taken. It is my understanding that FDA has recognized the potential risk and has recommended limiting the size of plasma pools. Therefore, I hope that today we hear from government and industry about a reasonable timetable to implement those recommendations.

But the issue of plasma pool size is only one of the reasons we are here today. The underlying fear driving questions about the size of plasma pools, the safety of the blood supply and the possible transmission of CJD has to do with the lack of action on AIDS. In 1993, the Department of Health and Human Services asked the Institute of Medicine (IOM) to study the spread of HIV through the blood supply in the early 1980's. In its 1995 report, the IOM concluded that "a failure of leadership and inadequate decision-making processes" led to the almost unrestrained spread of this fatal disease. As a result of a lack of governmental action in the early days of the AIDS outbreak, thousands of people may have needlessly suffered and died. The AIDS experience revealed the vulnerability of the blood supply and the weakness of the public health system. No one wants to see those kinds of mistakes happen again.

Therefore, the first question that we must ask here today is whether there are any similarities between CJD and AIDS. It is my understanding that available evidence suggests that CJD is not transmissible in humans through blood or blood products. Some recent evidence may suggest that the disease may be transmissible through blood in rodents. We should further examine that connection. However, we should note that the agent that causes CJD seems to

resist current methods used in the current purification process. Between 1983 and 1997, thirty-four reports were received and 37 donors were diagnosed with CJD. From the reports that we have received, it appears that these numbers are stable. However, we do not really know.

Mr. Chairman, we do not know because although we have given CDC the responsibility of collecting information on the appearance of new diseases and the growth of known diseases, we have failed to provide them with the resources to adequately perform that function. Although the CDC has some resources to track some diseases and is currently establishing active surveillance programs in six states, clearly we have not given them the funds they need to establish a comprehensive program which can track emerging or known diseases on a national basis. It is my understanding that the CDC is heavily dependent on voluntary collaboration with state and local health departments which in turn depend on physician reporting of a limited number of specific, recognized infectious diseases. Because people in every link along this voluntary reporting chain are more concerned with treating patients, these reports are often incomplete. Because federal resources are not provided to state and local health departments to support a national, active surveillance and notification system our ability to diagnosis and control infectious diseases is severely limited. While I share your concern about CJD as a future threat, I am also concerned about the current threat presented by tuberculosis, measles and other common diseases.

Mr. Chairman, if we learn anything from the AIDS epidemic, I believe we should learn the value of effective public health notification and active surveillance. I urge you to join with me in assuring that adequate funding for this kind of system is obtained.

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Mr. SHAYS. I have called our witnesses and they are at the table. As you know, we swear in everyone, including Members of Congress who come and testify. And at this time, I would ask you to stand and raise your right hand.

Let me also say if there is anyone on your staffs who you think might want to respond to a question, we will take their names if they do testify. But this way we don't have to swear them in twice or do it a second time. So if you all would raise your right hand.

[Witnesses sworn.]

Mr. SHAYS. OK. For the record, everyone standing answered in the affirmative.

This is a very important issue and one in which we appreciate having such expert witnesses. We will proceed in the order I called you: Dr. Satcher, then Dr. Brown and Dr. Zoon.

Dr. Satcher, we are going to try to stay within the bounds of 5 to 10 minutes. I will roll the 5-minute over, it will turn red, and then we will roll it over. But if we can stay close to 5, but if you go over a little bit that is all right.

STATEMENTS OF DAVID SATCHER, M.D., Ph.D., DIRECTOR, CENTERS FOR DISEASE CONTROL AND PREVENTION, ACCOMPANIED BY MARY CHAMBERLAND, BRUCE EVATT, AND LAWRENCE SCHONBERGER; PAUL W. BROWN, M.D., SENIOR RESEARCH SCIENTIST, LABORATORY OF CENTRAL NERVOUS SYSTEM STUDIES, NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE, NATIONAL INSTITUTES OF HEALTH; AND KATHRYN ZOON, Ph.D., DIRECTOR, CENTER FOR BIOLOGICS EVALUATION AND RESEARCH, FOOD AND DRUG ADMINISTRATION

Dr. SATCHER. OK. Thank you very much, Congressman Shays and members of the subcommittee. I am David Satcher, Director for the Centers for Disease Control and Prevention. I'm accompanied by Drs. Mary Chamberland, Bruce Evatt, and Lawrence Schonberger. We're pleased to be here this morning to discuss issues regarding plasma pool size and surveillance efforts related to Creutzfeldt-Jakob disease, CJD, and the blood supply.

The Nation's blood supply is safer than it's ever been. However, the blood supply continues to face infectious disease challenges from both recognized and unrecognized threats.

Since I last addressed the committee on this subject, in November 1995, CDC has implemented a number of steps to improve our ability to monitor and respond to potential threats to the blood supply. CDC has developed new and enhanced other surveillance systems. We have created a full-time position occupied by Dr. Chamberland to facilitate intra- and interagency coordination of CDC's blood safety activities. In addition, CDC continues to participate actively in various departmental and agency committees related to blood safety.

The risks for infectious diseases associated with plasma products have decreased dramatically since the introduction of donor screening and testing and effective viral inactivation procedures. Many viruses are efficiently inactivated. Unfailing adherence to and refinements of inactivation procedures, combined with donor screening, are our most critical safeguards for plasma products. However,

blood products made from plasma do carry risks for transmission of bloodborne infectious agents that cannot be eliminated through current inactivation practices. One strategy that has been proposed is to limit the number of individual donors who contribute to the large plasma pools.

The relationship between pool size and infectious disease risk is very complex and will not reduce infectious disease risk in certain situations. Nonetheless, CDC believes that setting an upper limit on the number of individual donors who contribute to pools used in the manufacturing of plasma products would be beneficial. Smaller pool size would provide an increased margin of safety to persons who receive infrequent infusions of plasma products. An industry-wide standard would have to be established. FDA is in the best position to work with industry to define an upper limit and determine how it can be implemented most expeditiously. It will be critical to ensure that our efforts to improve the safety of blood products do not result in interim product shortages.

Now concerning CJD, regarding potential transmissibility of CJD by blood and blood products, as Dr. Schonberger testified in January 1997, I reiterate CDC's assessment that the risk of transmission of CJD by blood and blood products is extremely small, if it exists at all.

The most direct reason for concern comes from experimental studies demonstrating the possible occasional presence of CJD agents in the blood of infected patients and the infectivity of blood when injected into animals. Some of these studies were conducted by Dr. Paul Brown from NIH who is on the panel today.

From animal studies we cannot directly infer that there is any risk of transmission of CJD by blood transfusion. To help answer these questions it is necessary to focus on available surveillance and epidemiologic data. CDC conducts routine surveillance for CJD through review of national mortality data that demonstrate stable annual rates for 16 years, from 1979 to 1995, and no case of CJD among persons with hemophilia. CDC also has undertaken to supplement its routine surveillance of CJD with an increased focus on persons with hemophilia.

Specifically regarding persons with hemophilia, CDC expanded its collaboration with hemophilia treatment centers by active solicitation for any case reports of CJD and by facilitating neuropathologic examination of brain tissue from deceased hemophilia patients to look for signs of CJD.

Finally, CDC is assisting the American Red Cross in coordinating a long-term study of persons who receive blood components from donors who are subsequently reported to have been diagnosed with CJD.

So how effective are CDC's surveillance efforts?

CDC is aware of two studies which indicate that routine mortality surveillance has good sensitivity for detecting CJD cases. One study found that 80 percent, another found 86 percent of confirmed CJD cases could be ascertained by review of death certificates.

Our efforts to supplement routine surveillance for CJD with focused activity in hemophilia treatment centers have had varying success—obtaining brain tissue from deceased hemophilia patients to examine for evidence of CJD is challenging.

CDC has developed a number of approaches to increase the level of participation by treatment centers and the number of brain autopsies performed on persons with hemophilia who die with neurologic disorders. CDC has begun direct funding of treatment centers in order to implement a nationally coordinated prevention program to reduce complications of hemophilia.

In these centers, we are phasing in our nationwide monitoring system, the Universal Data Collection System, which should capture bloodborne infections occurring in patients and improve participation in CJD surveillance activities.

So in conclusion, Mr. Chairman, ensuring the safety of the Nation's blood supply is an important public health priority and one to which CDC remains strongly committed to address. Enhanced surveillance can play an important role in helping to ensure the safety. Surveillance data have certain limitations and must be interpreted with caution; however, these data provide increasing support for CDC's conclusion that the risk of transmission of CJD by blood products in humans is extremely small and, in fact, remains theoretical. Periodic reevaluation of data will undoubtedly provide a stronger scientific basis for modifying public health procedures on CJD and blood safety in the future.

In regards to pool size, CDC concurs with FDA's proposal that some upper limit on pool size be established. We urge careful deliberation be undertaken by public health officials, by industry and consumers in advance of implementing pool size limitation to ensure that the supplies of these life saving products are not generalized.

It is a critical issue. Thank you for the opportunity to testify before the subcommittee. And I will be happy to respond to any questions.

Mr. SHAYS. Thank you, Dr. Satcher.

[The prepared statement of Dr. Satcher follows:]

Good morning. I am Dr. David Satcher, Director of the Centers for Disease Control and Prevention (CDC). I am accompanied by Dr. Mary Chamberland, Dr. Bruce Evatt and Dr. Lawrence Schonberger with CDC's National Center for Infectious Diseases. We are pleased to be here this morning to discuss with you issues related to blood safety; specifically, the safety implications of plasma pool sizes and our surveillance efforts related to Creutzfeldt-Jakob Disease (CJD) and the blood supply.

As the report published nearly a year ago by this subcommittee found, the Nation's blood supply is safer than it has ever been. However, the blood supply continues to face infectious disease challenges from both recognized, as well as unknown threats. Ensuring that the Nation's supply of blood and blood products is free of infectious agents is a public health responsibility shared within the Department of Health and Human Services (HHS) by CDC, the Food and Drug Administration (FDA), and the National Institutes of Health (NIH). While CDC has no regulatory responsibility for blood safety, as the Nation's Prevention Agency, it has the expertise and responsibility for surveillance and detection of public health risks associated with receipt of blood and blood products. In collaboration with FDA, CDC also keeps the public informed concerning such risks.

Since I last addressed the subcommittee in November 1995 on this subject, CDC has implemented a number of steps to improve our ability to monitor and respond to potential threats to the blood supply in the United States. Blood safety is a priority area in CDC's strategy to prevent emerging and reemerging infectious diseases. CDC has enhanced several of its surveillance systems, including those that monitor infections in persons who have hemophilia. CDC has also developed new surveillance programs, such as the system to detect bacteria-associated transfusion reactions that was described in a recent Morbidity and Mortality Weekly

Report (MMWR). This past year, CDC, in collaboration with FDA, responded rapidly to conduct a number of epidemiologic and laboratory investigations, including bacterial sepsis associated with contamination of intravenous albumin and possible exposure to porcine parvovirus among persons receiving porcine factor VIII concentrate. CDC has also created a full-time position, occupied by Dr. Chamberland, to facilitate intra- and interagency coordination of CDC's blood safety activities. In addition, CDC continues to participate actively in the PHS Interagency Working Group on Blood Safety; the HHS Blood Safety Committee, the FDA Blood Products Advisory Committee and Transmissible Spongiform Encephalopathy Advisory Committee, and the newly formed HHS Advisory Committee on Blood Safety and Availability.

You have asked me to address two specific issues related to blood safety. The first is the relationship between plasma pool size and the risk of infectious diseases; the second is the effectiveness of surveillance efforts to detect CJD in the blood supply and the experimental work that suggests the potential for transmission of CJD through blood products.

Infectious Disease Risks Associated with Plasma Pool Size

The risks for infectious diseases associated with plasma products such as albumin, immunoglobulins, and clotting factor concentrates, have decreased dramatically since the introduction of donor screening and testing, and most importantly, effective virus inactivation procedures. Viruses, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are readily and efficiently inactivated by such procedures. Since implementation of these inactivation procedures beginning in the mid-1980s, transmissions of HIV, HBV, and HCV by U.S. licensed products have been virtually eliminated in recipients of

clotting factor concentrates. Prior to this, clotting factor concentrates carried a substantial risk of transmitting HBV and HCV infection; and most tragically, nearly 10,000 persons with hemophilia were infected with HIV in the early 1980s. Unfailing adherence to chemical and physical inactivation procedures and their further refinement, combined with donor screening, are our most critical safeguards for plasma products.

Despite screening and viral inactivation techniques, blood products made from plasma carry risks for transmission of other bloodborne infectious agents. For example, clotting factor concentrates made from human plasma are still known to commonly transmit human parvovirus B19 and, in rare instances, hepatitis A. These infections are extremely resistant to the viral inactivation practices used in current manufacturing processes. In addition, the potential exists for other, as yet unknown agents, to be transmitted if their infectivity cannot be eliminated through current inactivation practices. One strategy that has been proposed is to reduce or limit the number of individual donors who contribute to the large pools of plasma that are used in the manufacture of these products. Typically, a single vial of a plasma derived product, such as immunoglobulin or clotting factor, contains plasma from more than 10,000, and perhaps as many as 400,000 or more different donors.

The relationship between pool size and infectious disease risks is very complex. On the one hand, limiting pool size is of little benefit for infections of high frequency in the donor population, and especially for persons who receive multiple or frequent infusions over a long period. In both of these situations, exposure to infectious pathogens that are resistant to inactivation would not be reduced. Also, use of larger pools is necessary for some products such as immunoglobulins, which require a minimum of 1,000 donors to ensure that persons receive an

adequate variety of antibodies. On the other hand, although the issue of pool size is relatively unimportant for known infections such as HIV, HBV, and HCV that are readily inactivated during processing, the large number of donors needed to produce these blood products automatically increases the risk for exposure to other infectious agents that are not inactivated. This is true for persons who receive a single or infrequent infusion (e.g., intramuscular immunoglobulin) -- a smaller pool size would reduce their risk of transmission of bloodborne infections. In addition, use of smaller volume pools by manufacturers could result in less disruption of supplies of blood derivatives and less expense in the event lot(s) of product are recalled, since fewer recipients would be involved.

CDC believes that setting an upper limit on the number of individual donors who contribute to pools used in the manufacturing of plasma products would be beneficial. Smaller pool sizes would provide an increased margin of safety to persons who receive single or infrequent infusions of plasma products. In addition, an industry-wide standard would be established. FDA is in the best position to work with industry to define an upper limit and determine how it can be implemented most expeditiously. If production practices require a large volume, one approach would be for manufactures to create pools made from repeat donations. This would decrease the number of donors in a pool without reducing the volume of the pool and the amount of available product, even after quality control testing. Whatever strategies or manufacturing changes are initiated, it will be critical to ensure that our efforts to improve the safety of blood products do not result in interim product shortages.

Creutzfeldt-Jakob Disease

In January 1997, Dr. Lawrence Schonberger summarized for the subcommittee available epidemiologic information about the potential transmissibility of CJD by blood and blood products. I would like to review the major points of his testimony as well as reiterate CDC's assessment that the risk of transmission of CJD by blood and blood products is extremely small, if it exists at all.

CDC understands the concern of the subcommittee and others about the potential transmissibility of CJD by blood and blood products. CJD is an invariably fatal brain disease that is caused by an unconventional agent. Disinfection is unusually difficult. Incubation periods are long -- measured in years; and there is no practical screening test to identify those who are incubating the disease. In addition, since the 1970s, cases of CJD associated with medical procedures, such as those caused by infected pituitary-derived growth hormone and dura mater grafts, have been increasingly recognized.

The most direct reason for concern about the risk of transmitting CJD by blood products are laboratory and experimental studies. These studies have demonstrated the possible, occasional presence of the CJD agent in minimal amounts in the blood of infected patients and have demonstrated the infectivity of blood throughout most of the incubation period in two different rodent models of CJD. Studies also have demonstrated the infectivity of the buffy coat (i.e., that part of blood that contains white blood cells and platelets), the plasma, and some derivatives from plasma, particularly cryoprecipitate, when injected into the brains of animals. In at least one study, CJD was transmitted in an animal model by intravenous inoculation of blood from a sick animal. Some of these studies have been conducted by Dr. Paul Brown from the NIH.

How closely animal models mimic human CJD, particularly the infectivity of blood throughout most of the incubation period, is not known; nor can we directly infer from them the risk, if any, of transmission of CJD by transfusion of blood and blood products. To help answer these critical questions, it is necessary to focus on available epidemiologic data.

First, CDC is aware of no compelling evidence for any instance of transmission of CJD to a human recipient by blood or blood products. The absence of such case reports contrasts to what happened in the United States in the 1980s, when reports first appeared describing transmission of CJD by pituitary-derived growth hormone and by a commercial brand of dura mater grafts. Further, the recent report from the United Kingdom of the possible spread of Bovine Spongiform Encephalopathy (BSE) to humans in the form of a new variant of CJD has increased physician and public awareness about CJD.

Second, case control studies are often the most practical epidemiologic studies for identifying risk factors for rare disease, such as CJD. The results of at least five such studies have consistently demonstrated that a history of a blood transfusion is not a risk factor for CJD. In none of these studies were patients with CJD more likely to have a history of blood transfusion than control subjects.

Third, CDC conducts routine surveillance for CJD through ongoing review of national mortality data. Results from 1979-1995 indicate that annual rates of CJD have remained stable (at about 1 case per million population). Thus, despite regular blood donation by persons who subsequently develop CJD, blood transfusions do not appear to be amplifying CJD infections in the U.S. population. None of the 3,905 reported cases of CJD was also reported to have hemophilia, thalassemia, or sickle cell disease -- diseases associated with increased exposure to

blood or blood products, such as clotting factor concentrates and/or cryoprecipitate. Because many such patients are exposed to blood products at a very early age, it is noteworthy that no CJD cases were reported in persons 5-19 years of age in the United States during this 17 year period.

Fourth, CDC has undertaken to supplement its routine surveillance for CJD with an increased focus on persons with hemophilia. As part of this effort, CDC expanded its collaboration with hemophilia treatment centers beginning in October 1995 by: 1) active solicitation of more than 140 centers for any case reports of CJD; and 2) facilitating neuropathological examinations of brain tissue from deceased hemophilic patients with neurologic disorders to look for signs of CJD and the presence of the agent thought to cause the disease. Despite active solicitation of treatment centers, as well as efforts to increase providers' awareness about CJD through educational symposia at national hemophilia meetings, no center has reported a patient with clinical CJD. CDC continues to make follow-up inquiries to the largest of these centers. Suitable autopsy material from 30 persons with hemophilia has been received to date. Neuropathological examination has been completed for 26 of these persons; none had evidence of CJD.

Finally, to further enhance the evidence derived from routine surveillance, CDC is assisting the American Red Cross in coordinating a long-term, follow-up study of recipients who received blood components from donors who were subsequently reported to have been diagnosed with CJD. Using primarily the national death index through 1995, the vital status of 178 recipients of transfusable blood components from 14 donors who subsequently developed CJD was determined; none of these recipients were reported to have died of CJD. Among these recipients,

41 persons lived 5 or more years after their transfusion, including nine who lived as long as 13 to 24 years.

Data from two additional sources have demonstrated similar findings. First, the Puget Sound Blood Center and Program in Seattle has shared with CDC followup data from a cohort of 101 persons who each received more than 100 units of cryoprecipitate for a bleeding disorder between 1979 and 1985. Cryoprecipitate is the blood component that contained the highest titers of the CJD agent in the recently reported animal model experiments. It has been estimated that several thousand persons with hemophilia nationwide have been treated with at least some cryoprecipitate; however, the Puget Sound patients were treated primarily with cryoprecipitate. Of these 101 persons, 76 remain alive a minimum of 11 to 17 years after receipt of cryoprecipitate; none of the 25 persons who have died were reported to have developed CJD. The second study was done in Germany; none of 27 patients who definitely, or eight who probably, received a unit of blood from a CJD donor died of CJD. At least 13 of these patients survived 10 years or longer after the transfusion.

You have asked that I specifically address the effectiveness of our efforts to detect CJD that may be related to receipt of blood and blood products. CDC is aware of two studies which indicate that routine mortality surveillance has good sensitivity to detect CJD cases. The first study, conducted in 11 states, found that 80 percent of all neuropathologically confirmed cases of CJD during 1986-1988 could be ascertained by review of death certificates. The second study was conducted in early April 1996 in four Emerging Infections Program sites in three states and two metropolitan areas as part of active surveillance for the newly reported variant of CJD and physician-diagnosed cases of CJD. In these surveillance areas, greater than 90 percent of all

pathologists, neurologists, and neuopathologists were contacted. Of the 94 CJD deaths identified during 1991-1995, 81 (86 percent) were found from death certificate review. These findings were reported in the CDC's MMWR on August 9, 1996.

Our efforts to supplement routine surveillance for CJD with focused activities in hemophilia treatment centers have met with varying success. The response to requests for reporting the number of patient deaths and patients who might have clinical CJD has been good. However, obtaining brain tissue from deceased hemophilia patients to examine for evidence of CJD has proved to be challenging. First, of the 140 Federally-funded hemophilia treatment centers that were invited to participate in this endeavor, only 52 volunteered. We attribute the modest level of participation to several reasons, including: 1) insufficient personnel and resources; 2) small size of some centers; 3) surveillance not being a routine function; and 4) CDC's direct funding of centers did not occur until October 1996, a year after initiation of the program in 1995. Second, we have received a small number of brain autopsy specimens, and most have been from persons who died before October 1995 when this program was initiated. According to records obtained from participating treatment centers, of 56 persons with bleeding disorders who died during January 1996-July 1997, 20 had central nervous system symptoms; of these 20, brain autopsy material from 6 (30 percent) persons has been identified to date. There appear to be a number of reasons for the small number of brain autopsies. Brain autopsy is not routinely performed on hemophilia decedents. Permission for an autopsy must be obtained during a time of family grief and high emotions. Hemophilia treatment staff have reported that it is difficult and uncomfortable to approach families of dying persons about consenting to a brain autopsy. A significant number of deaths occur outside of the hospital; consequently, hemophilia treatment

center staff often become aware of these deaths after it is too late to obtain material for examination. Finally, there is a reluctance on the part of pathologists to perform brain autopsy on persons known to be infected with HIV and possibly CJD.

CDC has developed a number of approaches to help increase both the level of participation by hemophilia treatment centers and the number of brain autopsies performed on persons with hemophilia who die with neurologic disorders. In October 1996, CDC began direct funding of hemophilia treatment centers in order to implement a nationally coordinated prevention program to reduce or eliminate the complications of hemophilia. CDC is in the process of fostering changes in the hemophilia treatment centers which will provide a needed surveillance and communication network. This will facilitate a quicker response time for investigation of reports of possible transmission of bloodborne agents. As part of this program, CDC is phasing in a nationwide monitoring system, the "Universal Data Collection System," which will collect information about the occurrence and severity of bloodborne infections among persons with bleeding disorders, provide free testing for bloodborne infections, and retain blood specimens to evaluate new agents that may threaten the safety of the blood supply. Implementation of the system, which has necessitated modification of many operating procedures in the 140 hemophilia treatment centers, is expected to be complete in 1998. When completely implemented, the system should capture nearly all bloodborne infections occurring in patients treated at Federally-funded hemophilia treatment centers and improve participation in the CJD surveillance activities. Until then, CDC is working hard to improve awareness and participation in these activities.

We believe that educating patients and physicians on the importance of the neuropathological examination component, and encouraging patients to enroll before they become

ill, will increase the number of autopsies. CDC plans to develop and distribute educational materials to hemophilia consumer and advocacy groups in addition to treatment centers regarding the CJD surveillance study. Additional efforts include working with pathologists to resolve concerns related to the handling of HIV-infected tissues and specimens and educating medical care providers about the importance of obtaining brain autopsies. Lastly, the implementation of the Universal Data Collection System may help recruitment for CJD surveillance. For example, the Universal Data Collection System informed consent process may facilitate discussions between care providers and patients about blood safety concerns, such as CJD, and lead to greater understanding and acceptance of the CJD surveillance project.

Conclusions

Ensuring the safety of the Nation's blood supply is an important public health priority and one to which CDC remains strongly committed to address. As part of its continued vigilance for emerging threats to the blood supply, CDC has a number of surveillance systems for the detection of diseases that may be transmitted in the blood supply. Surveillance is a critical component of CDC's mission. One of the four goals of CDC's strategic plan, "Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States," is the improvement and expansion of surveillance and response capabilities for infectious diseases in the United States and globally. Enhanced surveillance can play an important role in helping to ensure the safety of our blood and plasma products.

This morning, I have described CDC's surveillance and epidemiologic studies related to CJD and how we plan to strengthen these efforts. Surveillance and epidemiologic data have

certain limitations and must be interpreted with caution. They cannot establish the absence of a risk. However, surveillance and epidemiologic data from both the United States and other countries provide increasing support for CDC's conclusion that, despite some experimental evidence suggesting a potential for bloodborne transmission of CJD, the risk of transmission of CJD by blood products in humans is extremely small and remains theoretical. Periodic reevaluations of accumulating data will undoubtedly provide a stronger scientific basis for modifying, as appropriate, public health policies on CJD and blood safety in the future.

The current level of the safety of the blood supply largely reflects improvements in the areas of donor screening and education; serologic screening tests for viral pathogens; and viral inactivation techniques. The General Accounting Office, in its November 1996 report, Blood Supply: Transfusion-Associated Risks, indicated that new interventions will likely be of decreasing benefit and stated that new interventions will require careful consideration in order to identify areas of improvement that would maximize safety with reasonable costs. Limiting pool size is one intervention that could further improve the safety of blood products in some situations. However, compared to the aforementioned improvements that are already in place, the additional margin of safety to be gained is likely far less. We concur with the FDA's proposal that some upper limit on pool size be established. This would create a needed industry-wide standard. We urge that careful deliberation and study be undertaken by public health officials, industry, and consumers in advance of implementing pool size limitations to ensure that the supplies of these life-saving products are not jeopardized.

Thank you for the opportunity to testify before the Subcommittee. I will be happy to answer any questions you may have.

Mr. SHAYS. I know this room is very crowded, and I apologize for that. If there happen to be any people from the media, we have two chairs over there that I would have no problem being used. They may use those two chairs on that side and those two chairs there. I am welcome to have you do it.

Dr. Brown.

Dr. BROWN. Mr. Chairman, Mr. Pappas, Mr. Towns. Good morning and——

Mr. SHAYS. Thank you.

Dr. BROWN. And good morning and thank you for the opportunity to testify before you. My name is Dr. Paul Brown. I'm a board certified internist. However, I have spent the bulk of my life at the NIH studying issues related to the transmissible spongiform encephalopathies, notably Creutzfeldt-Jakob disease.

Any time that we transfer tissues or tissue extracts from one person to another, we have to be concerned lest we transfer some unwanted passengers. The recent outbreak of Creutzfeldt-Jakob disease in hypopituitary patients as a result of contaminated growth hormone, and the continuing occurrence of Creutzfeldt-Jakob disease in recipients of patients who have received dura mater grafts, warn us again to be vigilant about attending to the question of where risk might be preventable and trying to predict it and prevent it rather than simply cleaning up afterwards.

With this in mind, we have to ask the question, what is the risk, if any, of the recipient of a blood product or blood to contract Creutzfeldt-Jakob disease from that administration? That is not the same question as asking what is the probability of a patient with Creutzfeldt-Jakob disease contributing to a donor pool. The bottom line is what is the risk to an individual. And that is a three-step process.

And the first is: what's the probability that a CJD patient will donate blood to a blood pool?

The second step is: what is the probability that such a donation will in fact be contaminated? Is there going to be infectivity in the blood?

And the third step is: in a recipient exposed to blood that does have the infectious agent in it, what is the probability that that person will be, in fact, infected?

Each one of these steps is contributed to by a number of things which we will not have time this morning to go into. Blood pools and the size of blood pools contribute to the first two steps. Clearly, it's a matter of common sense to say that if a disease like CJD has a prevalence of about one in a million, that a pool size of a million people will have a much greater chance of being contributed to by a person with CJD than if the pool size is 10,000. It's just common sense.

The numbers for a pool size of 10,000, 100,000, 500,000 are in the written statement. But in general, if we take the one in a million prevelance figure, a pool size of 10,000 would have a probability of a little less than 1 percent of being—of being contaminated, had being contributed to by a CJD donor and a pool size of 100,000 about 7 percent. If you push it up to 500,000, the probability that a CJD donor is going to be amongst those contributors goes up to 20 to 30 percent.

Now you'd say that's not good and, therefore, we should keep pool sizes small. However, if there are three donors out there, it really doesn't matter whether they donate to one pool or five pools. The same number of donors are going to be contributing. And, therefore, it doesn't much matter whether you've got 10 pools of 10,000 contributors or 1 pool of 100,000 contributors. The same number will be there.

Now, you might say, well, wouldn't it be better if the contamination was only the three pools? We'd have at least seven pools that we knew were clean. And that is quite correct. But that brings us to the second step, which is how much infectivity is going to be found in the blood of a donor. And CJD is a little special in that regard, because unlike hepatitis or AIDS, the amount of infectivity in the blood of a CJD donor, although we don't have precise measurements, is almost certainly very small. And unlike the situation with HIV, a single donation could not saturate the entire donor pool. With CJD we're probably talking, at most about 10, 20, 30, 40 infectious particles. And they will be fully dispersed in donor pool sizes as small as 10,000 donors. So that those 30 or 40 particles are still going to find their way to 30 or 40 different recipients, whether the pool has 10,000, 50,000, or 100,000 donors.

And that brings us to the third step, which is what's the likelihood that a patient who's getting a product is going to be infected. Again, we don't know the answer to that question with precision. We do know that the administration by peripheral routes, as opposed to intracerebral inoculation directly into the brain, is a very inefficient way of transmitting infection. This is not an easy disease to get. We know that the efficiency is anywhere between 10 and 10,000fold less. So the question as to whether or not a person is actually going to contract CJD from contaminated blood donation is not at all clear.

I think this morning, in conclusion, you will certainly have a consensus, if for no other reason than the common sense reason, that if you decide to recall a pool, it certainly seems to make sense to recall a smaller pool than a larger pool. But I would hope that the committee and the general public through this committee would recognize the equal importance of what you said in your preamble, which is that continuing research is needed on the questions for which we still have very imperfect information. How long, for example, before a CJD patient develops signs, is his blood infectious? How inefficient is intravenous administration of a product? Can we clean up the plasma in ways that would be quite simple? For example, why not spin plasma 10 times faster or 5 times longer if infectivity of this disease is associated with white cells? What a simple way to clean up plasma. We just sediment the infectivity. Nobody has done it. What if we used iodine? We have a collaboration now with Dr. William Drohan in the Red Cross which gives us a possibility of perhaps inactivating the virus. So these are the kinds of laboratory experiments that should throw some light on the problem. And I very much look forward at this sort of twilight of my own career to be in a position to help solve some of these problems.

Thank you, Mr. Shays.

Mr. SHAYS. Thank you. I am tempted to ask you to define twilight in your career.

Dr. BROWN. Pink scalp.

Mr. SHAYS. That would apply to many. And I'm not offended.

[The prepared statement of Dr. Brown follows:]

Mr. Chairman and members of the subcommittee, good morning, and thank you for inviting me to speak to you about the potential risk of transmitting Creutzfeldt-Jakob disease (CJD) via blood or blood products. My name is Dr. Paul Brown; I am a Board Certified Internist who has spent most of his professional career at the National Institute of Neurological Disorders and Stroke investigating various aspects of a group of diseases known as the transmissible spongiform encephalopathies, of which CJD is the best known example.

CJD is a fatal degenerative disease of the brain that mainly affects adults between the ages of 50 and 75 years. It usually begins with a loss of memory that gradually progresses to frank dementia, and is accompanied by physical deficits such as incoordination, slurred speech, visual loss, muscle twitching (myoclonus), rigidity, weakness, mutism, and coma. The entire process is not unlike Alzheimer's disease run in "fast forward," and plays out to an invariably fatal ending in less than a year's time from the onset of symptoms.

Left to its own devices, CJD afflicts only about one in a million people each year, which translates to about 250 cases in the United States, a figure that exceeds our present day experience with polio or rabies but falls short of the concerns presented by AIDS, viral hepatitis, herpes infections, or even measles. Why should this comparatively rare disease be the subject of so much attention? Most likely, because it shares so many features of the numerically more important Alzheimer's disease but is even more devastating to witness, and because it can be transmitted through medical procedures that often involve young people.

A poignant recent example of medically caused CJD is the outbreak of disease in hypopituitary patients treated with native growth hormone that until 1985 was extracted and processed from the pituitary glands of cadavers. Some cadaver donors had unsuspected CJD, and their glands were included in random batches of hormone used to treat some 7000 plus patients in the United States. The resulting contamination is responsible, to date, for 21 deaths, that, together with the consequences of similar contaminations in England and France, account for a total of nearly 100 deaths in treated adolescents and young adults, with new cases continuing to occur after longer and longer incubation periods following infections that occurred in the 1970's. Advances in biotechnology supported by the NIH have made possible an unlimited supply of recombinant growth hormone free of the risk inherent in the use of growth hormone of human origin.

The growth hormone tragedy, and an even more recent outbreak of CJD in neurosurgical patients who years ago had received contaminated dura mater grafts - also from cadaver donors

who had unsuspected CJD - has forced us to consider with renewed concern any medical procedure that involves the transfer of tissue or bodily fluids from one human being to another, and to try to predict where further danger might be present and preventable.

This morning we are considering the possible risk that an individual receiving blood (or a blood product) will contract CJD. The degree of this risk depends upon a sequence of three probabilities: the probability that pooled blood will contain a donation from at least one individual with CJD, the probability that an individual receiving a therapeutic product made from such a pool will be exposed to the infectious agent, and the probability that a recipient who is so exposed will be infected and contract CJD.

The probability that pooled blood will contain a donation from a diseased individual depends the prevalence of CJD in the donor population, and the number of donors who contribute to the blood pool (donor pool size). The prevalence of CJD in the United States is estimated to be one case per 1.3 million people, and the size of donor pools in current practice typically ranges from a low of 10,000 donations to a high of 100,000 donations, although an occasional pool reaches 400,000 donations. Using the 10,000 to 100,000 numbers, the probability that a CJD patient will contribute to a pool of 10,000 donors is 0.8%; if the pool size is increased to 100,000 donors, the probability rises to 7.6%.

Because it is unusual for a person who is already ill with CJD to donate blood, our primary concern should be directed towards donations made during the period before illness begins. Unfortunately, we do not know how long blood might be infectious before a person shows the symptoms of CJD, but from studies in experimental animals, we can make an educated guess that infectivity could be present for as long as 10 years before the onset of symptoms. The prevalence figure for a person "incubating" CJD would thus be 10-fold greater than the prevalence of clinically apparent CJD, and the probability that a potentially infectious individual would contribute to a donor pool becomes 7.6% for a pool of 10,000 donors, and 55% for a pool of 100,000 donors.

The next step in the risk sequence - the probability that a recipient of blood from a pool to which a CJD patient had contributed will be exposed to the infectious agent - depends upon the amount of the infectious agent in the donor pool, the number of particles of the agent needed to produce an infection, and the number of recipients. Three different situations are possible.

First situation: the donor pool contains a large number of infectious particles.
For example, if a donor pool contains 10,000 infectious particles, the pool is more or

less "saturated": if given to a single recipient, one infection will result; if divided among 100 recipients, it is extremely likely that 100 infections will result, etc., until the number of recipients approaches several thousand, when the increasing dilution and random distribution of infectious particles will start to spare some recipients. This kind of situation occurs in AIDS, where the blood of an HIV-infected donor may contain up to 100,000 infectious particles of virus.

Second situation: the donor pool contains a small number of infectious particles. For example, if the pool contains 5 infectious particles, and the donation is given to a single recipient, one infection will result; if divided among 10 recipients, between one and five infections will result (a statistical probability calculation). If the donation is divided among 100, or 1000, or 10,000 recipients, five infections will almost always result, as distribution randomness will make it highly probable that each infectious particle goes to a separate recipient. This is the most likely situation for CJD, based on unpublished data from experimentally infected animals, but levels of infectivity in the blood of human patients with CJD have never been determined.

Because we do not know with certainty what number of particles are needed to produce an infectious unit, we must also consider the possibility that two or more particles must join together to make a single infectious unit (for example, if the required number were two, a specimen containing 100 particles would contain 50 infectious units)

Third situation: one infectious unit consists of two interactive particles that are independently distributed physical entities. For example, if a pool containing 200 particles (100 infectious units) is given to 10 recipients, all recipients are likely to be infected. If it is divided among 100, 1000, or 10,000 recipients, the random distribution of particles will result in fewer and fewer individuals receiving the necessary two particles, and a progressively decreasing number of infections will be observed, eventually reaching zero. Thus, increasing the size of the donor pool would "dilute out" its infectivity.

The final step in the risk sequence - the probability that a recipient who is exposed to the infectious agent of CJD will be infected - depends upon the ease with which the agent can be transmitted by intravenous or intramuscular administration. We do not know the answer to this question in humans, but in experimental animals, these types of peripheral inoculations are 10 to 100 times less effective than direct intracerebral inoculations in transmitting disease.

In summary, we conclude that although mathematical modeling gives us a fairly precise idea of the influence of pool size upon risk of exposure, estimates of the actual risk of a recipient contracting CJD from a contaminated blood product are impaired by our lack of knowledge about most of the biological factors that contribute to such estimates. Considering the magnitude of the variables involved (low prevalence of CJD in the general population, low levels of infectivity in the blood of CJD patients, and low efficiency of disease transmission by intravenous or intramuscular administration), it seems likely that the chance of contracting CJD from a pooled blood product to which a patient with CJD has contributed is extremely small, no matter what the size of the donor pool. The fact that epidemiological studies have so far been unable to identify a single case of CJD resulting from the administration of blood or blood products supports this contention.

Appendices

Brown, P. Can Creutzfeldt-Jacob Disease Be Transmitted by Transfusion? Current Opinion in Hematology 1995, 2:472-477

Abstract of manuscript

Summary of Future Research Proposals

APPENDIX 1

Can Creutzfeldt-Jakob disease be transmitted by transfusion?

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The transmissible agent of Creutzfeldt-Jakob disease, a dementing neurodegenerative disorder, is present in many tissues of the body, even though its pathologic consequences are confined to the brain. Experimental animal models of the disease have shown that blood (most probably the leukocyte component) can be infectious in both the clinical and preclinical incubation stages of the disease, and there are also a few reported isolations of the agent from whole blood, buffy coats, or serum from humans with Creutzfeldt-Jakob disease. Despite this potential for blood-borne iatrogenic infection, epidemiologic studies do not support the contention that the administration of blood, blood components, or blood derivatives transmits the disease; in particular, not one of nearly 2000 patients who have been studied during the past two decades has been shown to have acquired the disease from a blood donor who later died of Creutzfeldt-Jakob disease. This fact does not diminish our responsibility to preclude such an occurrence from happening in the future, and will require an unremitting effort to screen from the blood donor population all individuals with a higher than average risk of harboring the infectious agent; namely, donors with neurologic disease, a family history of neurologic disease, or a history of events that have been identified as leading to iatrogenic Creutzfeldt-Jakob disease, such as neurosurgical procedures involving dura mater homografts or treatment with native pituitary hormones.

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The question posed by the title of this paper should really be bisected into two related questions with different implications: 1) *can* blood transfusion transmit Creutzfeldt-Jakob disease (CJD)? and 2) *does* blood transfusion transmit CJD? For those readers unfamiliar with the disease, CJD is a fatal neurodegenerative process that occurs in sporadic, familial, and iatrogenic forms; in its sporadic form (which accounts for approximately 90% of cases), it primarily affects people in late middle age as a rapidly progressive dementia associated with a battery of neurologic abnormalities that typically include cerebellar signs (ataxia, incoordination, and dysarthria), involuntary movements (especially myoclonus), and characteristic periodic triphasic 1- to 2-cycle per second electroencephalogram activity, leading to death in less than 6 months [1••]. Clinical patterns can vary in familial forms of the disease, and in peripherally infected patients with iatrogenic CJD, the illness has a distinctive, predominantly cerebellar character, with little or no dementia. Pathognomonic neurohistologic abnormalities consist of a diffuse vacuolation (spongiform change) of the gray matter associated with variable degrees of gliosis and neuronal loss.

Transmissibility of the disease has been established both experimentally [2] and as a consequence of iatrogenic misfortunes in which contaminated surgical instruments, homografts, or pituitary tissue extracts have been incriminated as sources of infection [3]. Although only the central nervous system is affected pathologically, the infectious agent is present in a wide variety of body tissues, so that the first question that must be addressed is whether blood or blood products might be infec-

Abbreviation
CJD—Creutzfeldt-Jakob disease.

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tious, and so have the potential to transmit disease. Before reviewing the evidence, a word is necessary about the methodology.

As with many infectious diseases, the most sensitive method of detection has been, and continues to be, disease transmission from human tissue to susceptible experimental animals; in the case of CJD, the most sensitive animals are nonhuman primates, but transmission has also been accomplished in a variety of laboratory rodents, including mice, hamsters, and guinea pigs. The most accurate procedure is to prepare a series of dilutions of a suspension of the tissue in question, inoculate them intracerebrally into groups of the chosen experimental animal species, and then keep the inoculated animals under surveillance for a period of months to years, examining all dead animals for the histopathologic signs of spongiform encephalopathy. The amount of infectivity is then calculated by the Reed-Muench statistic based on the ratio of infected to total inoculated animals in each dilution group (end point dilution), and is expressed as a mean 50% lethal dose (LD_{50} per unit of inoculated tissue).

Because this is a costly and time- and space-consuming method in studying a disease that may take years to transmit, a short-cut has been devised that provides an estimate of infectivity based on its relationship to the interval between inoculation and onset of disease (incubation period): the longer the incubation period, the less the infectivity. In this assay, a single dilution of the specimen (usually 10^{-2} or 10^{-3}) is inoculated into a group of animals, and the resulting average incubation period is compared with those of a dilution series having known amounts of infectivity; the amount of infectivity in the tested specimen is then interpolated from this curve.

A considerable body of information about the infective potential of blood and blood components has accumulated from studies in animals infected with the infectious agent of scrapie, an analogous disease of sheep and goats that has also been adapted to laboratory rodents [4–13] (Table 1). In naturally infected sheep and goats, infectivity has never been detected in blood; however, in experimentally infected laboratory rodents, infectivity in blood or blood constituents has been detected independently in a number of different laboratories, both from animals that are clinically ill and from animals in the preclinical, incubation phase of disease. Because the infective agent is associated with the plasma cell membrane of nucleated cells, it appears highly likely (although not proven) that the infectivity is solely attributable to the leukocyte component of blood, as is also suggested by two studies of experimental CJD in which buffy coats from a large number of guinea pigs and mice were found to be infectious throughout the course of disease [14,15]. Serum infectivity has been noted in three reports, but none demonstrably excluded

Table 1. Attempts to detect infectivity in the blood of animals with scrapie or Creutzfeldt-Jakob disease

Study	Host animal	Inoculated material	Assay animal	Route of inoculation	Transmissions/total inoculated specimens, *n/n**
Scrapie (natural)					
Hadlow *et al.* [4]	Goat	Blood clot/serum	Mouse	ic	0/3
Hadlow *et al.* [5]	Sheep†	Blood clot/serum	Mouse	ic	0/18
Scrapie (experimental)					
Pattison and Millson [6]	Goat†	Whole blood	Goat	ic	0/14
Gibbs *et al.* [7]	Sheep	Serum	Mouse	ic	1/1
Clarke and Haig [8]	Rat	Serum	Rat	ic	1/1 (pool)
	Mouse	Serum	Mouse	ic	1/1 (pool)
Eklund *et al.* [9]	Mouse†	Whole blood	Mouse	ic	0/39
Dickinson and Meikle [10]	Mouse†	Whole blood	Mouse	ic	3/13
Hadlow *et al.* [11]	Goat†	Blood clot	Mouse	ic	0/3
				sc	0/20
Diringer [12]	Hamster†‡	Extracted blood	Hamster	ic	5/5 (pools)
Casaccia *et al.* [13]	Hamster†	Extracted blood	Hamster	ic	10/11 (pools)
CJD (experimental)					
Manuelidis *et al.* [14]	Guinea pig†	Buffy coat	Guinea pig	ic, sc, im, ip	10/28 (pairs)
Kuroda *et al.* [15]	Mouse†	Buffy coat	Mouse	ip	4/7 (pools)

*Infectivity level (estimated by incubation periods in assay animals) in all of the tested blood donor materials was ≤100 LD_{50}.
†Samples taken during incubation and clinical phases of disease.
‡No detectable infectivity in unextracted whole blood.
CJD—Creutzfeldt-Jakob disease; ic—intracerebral; im—intramuscular; ip—intraperitoneal; sc—subcutaneous.

leukocytes (or cell fragments) from the centrifuged serum.

The situation in humans is somewhat less clear-cut than in experimentally infected animals (Table 2). The National Institutes of Health Laboratory of Central Nervous System studies was uniformly unsuccessful in transmitting disease from the blood of 13 patients with CJD, using either highly susceptible primate hosts (nine cases, including whole units of blood from three cases that were transfused into chimpanzees) or less susceptible rodent species (four cases) [1••]. In contrast, four other laboratories have reported infectivity to be present either in whole blood or buffy coat from four patients with sporadic CJD and one patient with iatrogenic CJD due to treatment with contaminated native growth hormone [16,17,18•,19]. Each of these studies merits a brief comment.

Tateishi [16] isolated the infectious agent from the blood of one of three patients with sporadic CJD, as well as from the urine, cornea, and brain of the same patient. The finding of infectivity in urine is unique, despite repeated attempts in both humans and experimentally infected animals, and a comparison of incubation times in the animals inoculated with the different specimens suggests that the urine contained a higher level of infectivity than either the blood or cornea, and not too much lower than that of the brain, which is surprising. The author was unable to confirm this urine isolation in a subsequent experiment (Tateishi, Personal communication).

Tamai *et al.* [17] observed infectivity in 3X concentrated plasma from a pregnant woman with CJD, as well as in brain, placenta, umbilical cord leukocytes, and colostrum, but not in the patient's unconcentrated plasma or leukocytes, or from umbilical cord plasma or amniotic fluid. This pattern of isolations is peculiar and a comparison of incubation periods in animals inoculated with the positive specimens suggests that the level of infectivity in plasma approximated that in brain, which is virtually impossible.

Deslys *et al.* [18•] found infectivity in the buffy coat of a single patient with growth hormone–related CJD. Only one of five inoculated hamsters became ill, after a comparatively long incubation period of 20 months, and the other four inoculated hamsters, as well as three of the five uninoculated control hamsters, died from unknown causes during this period and were cannibalized and so unavailable for examination. Although none of these facts disproves the lone positive transmission, its long incubation period and the very high unexplained mortality in both inoculated and control groups raise the question of whether the transmission might possibly have been an artifact of laboratory animal cross-contamination.

Table 2. Attempts to detect infectivity in the blood of humans with Creutzfeldt-Jakob disease, kuru, and Alzheimer's disease, in relatives of people with Alzheimer's disease, and in normal subjects

Study	Diagnosis	Inoculated material	Assay animal	Route of inoculation	Transmissions/total inoculated specimens, *n/n*
Manuelidis *et al.* [19]	Sporadic CJD	Buffy coat	Guinea pig, hamster	ic	2/2
Tateishi [16]	Sporadic CJD	Whole blood	Mouse	ic	1/3
Tamai *et al.* [17]	Sporadic CJD	Concentrated plasma*	Mouse	ic	1/1
Brown *et al.* [1••]	Sporadic CJD	Whole blood	Chimpanzee	iv	0/3
	Sporadic CJD	Whole blood	Spider monkey	ic, iv, ip	0/1
	Sporadic CJD	Whole blood	Squirrel monkey	ic, ip, im	0/1
	Sporadic CJD	Buffy coat	Squirrel monkey	ic, iv	0/4
	Sporadic CJD	Whole blood	Guinea pig	ic, ip	0/1
	Kuru	Serum	Mouse	ic	0/3
Deslys *et al.* [18•]	hGH iatrogenic CJD	Whole blood	Hamster	ic	1/1
Manuelidis *et al.* [20]	Familial AD	Buffy coat	Hamster	ic	1/2
Manuelidis and Manuelidis [21]	AD relatives				4/9
	Normal subjects				26/30
Godec *et al.* [22,23•]	Sporadic AD	Buffy coat	Hamster	ic	0/4
	Familial AD				0/8
	AD relatives				0/30
	Normal subjects				0/22

*No detectable infectivity in unconcentrated plasma or in leukocytes.
AD—Alzheimer's disease; CJD—Creutzfeldt-Jakob disease; hGH—human growth hormone; ic—intracerebral; im—intramuscular; ip—intraperitoneal; iv—intravenous.

Manuelidis *et al.* [19] isolated the infective agent from the buffy coat of each of two patients with sporadic CJD; buffy coat from one patient transmitted disease to one of four guinea pigs (three died of unrelated causes), and the other transmitted to two of five hamsters (three died of unrelated causes), but not to any of five guinea pigs. As in the study by Deslys *et al.* [18•], this very high non-CJD experimental mortality is disquieting. In subsequent experiments, the same authors reported transmission of CJD to hamsters from the buffy coats of several patients with preclinical or early Alzheimer's disease, and from 26 of 30 healthy control individuals [20,21]. Their explanation for these startling results was that the infectious agent exists in the human population in a variety of strains with differing virulence which, in combinations with undetermined environmental or host factors, determines susceptibility to disease. The National Institutes of Health Laboratory of Central Nervous System studies was unable to verify these results in duplicate experiments [22,23•], and an alternative explanation has been proposed: CJD was mistaken in the inoculated hamsters for an endemic late-onset disease caused by *Clostridium difficile*, coupled with laboratory cross-contamination by the agent of spongiform encephalopathy in second passage experiments [24].

These remarks are not meant to disparage the authors or to imply that the reported isolations from blood are necessarily invalid; indeed, the fact that transmission of CJD from blood components has apparently been accomplished by four different laboratories lends credence to their validity. However, one or more aspects of each report are puzzling because they are inconsistent with what we know about the distribution and amount of infectivity in tissues of experimentally infected animals and invite a more cautious appraisal than might be accorded by the casual reader.

In summary, then, the answer to the question "*Can* blood transfusion cause CJD?" is a qualified "yes," based on the fact that blood (and especially buffy coat) in experimental animal models of CJD contains comparatively small amounts of the infectious agents, and bolstered by reported isolations of the infectious agent from the blood of five humans with CJD. The more important question "*Does* blood transfusion cause CJD?" is problematic.

On the experimental side, it is important to note that all but one of the transmissions from animal blood and all transmissions from human blood have resulted from the highly efficient but eminently artificial method of intracerebral inoculation; the only three attempts to transmit disease by intravenous transfusion all failed. Thus there exists no direct experimental confirmation of disease transmission by blood transfusion, and all other evidence bearing on the question comes from epidemiologic studies of blood donors and recipients in the CJD patient population.

Four separate epidemiologic case control studies conducted during the 1980s in the United States [25,26], the United Kingdom [27], and Japan [28] all found the percentage of patients with CJD who had received blood transfusions to be no higher than in healthy control groups. The UK study also found no increase in the incidence of CJD in areas where multiple blood donations from CJD patients had been distributed. Newer case control studies have been incorporated into the ongoing program of CJD surveillance in several European nations, and data analyzed from the first 3-year period again find no significant differences between CJD and control populations in the proportion of individuals who had received blood (Will, Personal communication). Although a recent report from Australia described four patients who had received transfusions 5 years before the onset of iatrogenic-type CJD illnesses (cerebellar signs with little or no dementia), no information was provided about the comparable frequency of transfusions in a non-CJD control population or about the existence of CJD patients among the blood donors [29].

A different approach was taken in Germany, where an effort was made to trace all recipients of blood from a regular donor who later died of CJD [30•]. Among 35 of 55 identified recipients, 21 had died from non-CJD illnesses up to 22 years after having received transfusions, and 14 recipients were still alive without evidence of neurologic disease after an average survival of 12 years. Similar efforts are being pursued for several more such incidents by the American Red Cross and the European CJD surveillance study. Notable findings include the identification of a US patient who had donated a total of 93 units of blood during the 35-year period before the onset of CJD and several members of a French family with CJD who also had donated blood on multiple occasions.

Finally, it is worth recalling that no case of CJD has ever been identified among patients whose diseases require (or required before recombinant technology) repeated administration of whole blood, blood components (*eg*, plasma or leukocytes), or blood derivatives (*eg*, albumin, immune globulin, leukocyte interferon, α_1-antitrypsin, and clotting Factors VIII and IX). Such potentially high-risk groups include patients with hemophilia, immune deficiency or suppression syndromes, mul-

tiple sclerosis, α_1-antitrypsin deficiency, and multiple surgical procedures.

None of these observations is definitive, because population-based studies can never prove or disprove the possibility that rare cases might result from blood transfusions but be numerically insufficient to produce a statistically significant difference from case control groups; and single-incident studies, even if all recipients are traced (which is almost never possible), may still be criticized because a proportion of recipients will have died from other illnesses before CJD has had a chance to manifest itself. On balance, the available evidence favors the conclusion that blood-borne infection is not a cause of CJD, but there nonetheless remains an undeniable if very small potential risk of iatrogenic disease from blood and blood products, and our best defense against the risk is an unremitting vigilance in screening from the donor population all individuals with neurologic disease, a family history of neurologic disease, or a history of events that have been identified as leading to iatrogenic CJD, such as neurosurgical procedures involving dura mater homografts or treatment with native pituitary hormones. This rigorous screening procedure should eliminate virtually all individuals at higher than usual risk of incubating the transmissible agent of CJD; it will not eliminate the one per million healthy donors who in any given future year will die from sporadic CJD, but the solution to this problem must await the discovery of a preclinical marker of disease.

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Appendix 2 - abstract of manuscript (May, 1997)

Evaluation of the potential risk of iatrogenic Creutzfeldt-Jakob disease from the administration of blood, blood components, or plasma derivatives donated by patients later dying of Creutzfeldt-Jakob disease

Background information. Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy that occurs in the general population at an annual frequency of about 1 case per million people. Approximately 90% of cases are sporadic, without known cause, and most of the remaining 10% of cases are inherited as a consequence of mutations in a gene on chromosome 20 that encodes a potentially amyloidogenic protein (PrP). However, there is also a small but increasing number of iatrogenic cases due to infection from contaminated neurosurgical instruments, homografts (cornea, dura mater), or tissue extracts (cadaver derived pituitary hormones).

CJD is related to scrapie, a disease of sheep known to be infectious for the past 50 years, subsequently adapted to laboratory rodents for purposes of experimental study. CJD was first transmitted experimentally to non-human primates in 1968, and has also been transmitted and adapted to numerous rodents. Experimental data from studies of both scrapie and CJD have shown that the CNS (which is the only pathologically affected tissue) contains high concentrations of the infectious agents (up to 10^{10} LD_{50}/g), and that other tissues, including blood, may also be infectious throughout both the preclinical and clinical phases of disease, but at much lower concentrations (less than 10^{3} LD_{50}/ml).

Because the majority of human cases of CJD have no known antecedent cause of infection, and therefore no defined pre-clinical incubation period, blood donated by such individuals both during the course of their illness, as well as during the years before the onset of their illness, has the potential to be infectious. Epidemiological studies have shown that 10-20% of patients dying of CJD have donated blood at some point during their lifetimes, often during the years just preceding the onset of CJD. Thus, in the US, where some 250 cases of CJD are diagnosed each year, it is reasonable to expect that perhaps 20 to 30 of them have donated blood. Although no case of iatrogenic disease from blood or blood products has ever been proven, the potential risk from this source of contamination is drawing increasing attention by the National Hemophilia Foundation, the therapeutic plasma product industry (including the American Red Cross), the FDA, and most recently, Congress.

We recently completed a preliminary study in which mice infected with the Fujisaki strain of human CJD were exsanguinated at the onset of illness; the blood was separated into its components and plasma was processed into Cohn fractions. We found low levels of infectivity in buffy coat, plasma, cryoprecipitate (the source of Factor VIII and fibrinogen), and Cohn fraction I+II+III (the source of gamma globulin). Infectivity was not detected in Cohn fraction IV (the source of a_1 anti-trypsin and Factor IX complex), or Cohn fraction V (the source of albumin).

Appendix 3 - Proposed Future Experiments

The presently proposed set of experiments is designed to answer 3 questions raised by this earlier experiment:

1) Is infectivity present in plasma during the incubation period?
2) Is infectivity detectable when assayed by intravenous inoculation?
3) Is infectivity in plasma the result of incomplete buffy coat separation?

The Fujisaki strain of CJD will be inoculated into 300 mice. At 4, 8, and 12 weeks after inoculation, groups of 25 mice will be exsanguinated, and the blood separated using the ARC protocol into red cell, white cell/platelet, and plasma components, which will be stored at -70°C until assayed (Answer to Question 1). When the remaining mice begin to show signs of disease, about 16 weeks after inoculation, they will be exsanguinated and a portion of the blood similarly separated and stored for parallel infectivity assays using intracerebral and intravenous inoculation routes (Answer to Question 2). The remaining blood will be divided in two portions, each of which will be separated into its white cell/platelet and plasma components using two different centrifugation velocities before storage for infectivity assay (Answer to Question 3). Note that the 16 week ARC-separated blood assayed by intracerebral inoculation can be used for comparative purposes in each of the three experiments.

Animals will be kept under **observation for 1 year**, and the brains from all animals, whether dying during the observation period or alive at the conclusion of the experiment, will be examined by Western blot for the presence of the pathognomonic prion protein.

In a parallel study, we propose to inoculate animals with buffy coat, plasma, and cryoprecipitate from several human patients with classical CJD, "new variant" CJD (thought to have resulted from exposure to bovine spongiform encephalopathy), and from mutation-positive but members of CJD families in the pre-clinical stage of disease. We have obtained specimens from the CJD Epidemiological Surveillance Unit in the United Kingdom, and will inoculate them in parallel with specimens already in our possession from patients with sporadic and familial forms of CJD.

From each patient, buffy coat and cryoprecipitate will each be inoculated into 25 mice, and plasma will be inoculated into 50 mice (i.e., 100 mice per patient). Transgenic as well as wild type mice will be used. Animals will be kept under **observation for 2 years**, and the brains from all animals, whether dying during the observation period or alive at the conclusion of the experiment, will be examined by Western blot for the presence of prion protein.

The same series of specimens will be inoculated intracerebrally into pairs of squirrel monkeys, and selected specimens will be inoculated intravenously into chimpanzees. Animals will be kept under **observation for at least 3 years**.

Mr. SHAYS. Dr. Zoon.

Ms. ZOON. Thank you.

Mr. Chairman and members of the committee, I am Dr. Kathryn Zoon, Director of the Center for Biologics Evaluation and Research of the Food and Drug Administration. I appreciate this opportunity to discuss the safety of the blood supply and the safety of plasma derivatives.

Mr. Chairman, you requested that we address safety implications of plasma pool size in the manufacture of plasma derivatives. Let me start by stating that the FDA believes that upper limits on plasma pool size will have public health benefits. It is, however, only one of the aspects of FDA's commitment to blood and blood product safety. And I will discuss it in more detail later in my testimony.

FDA is absolutely committed to taking every appropriate action to help ensure the safety of the Nation's blood supply. In recent years, we have taken numerous steps in this regard. I would like to briefly explain some of those initiatives.

As you will recall, Mr. Chairman, these efforts were elaborated on by Dr. Friedman's testimony to this committee last month. For example, some of these initiatives by FDA have focused on good current manufacturing practices, or GMPs, and FDA expects these to be a primary concern to the manufacturers of blood and plasma-derived products. To ensure substantially greater attention to this issue, the lead responsibility for conducting inspections of plasma fractionators has been transferred from the Center for Biologics to the Office of Regulatory Affairs.

The Center for Biologics Evaluation and Research's internal emergency response procedures have been redesigned to assure a more effective and coordinated response to emergency situations.

FDA has provided enhanced public access to recalls and withdrawals of plasma derivatives by providing easily accessible information through the Internet, fax, and e-mail.

FDA now receives monthly reports from plasma derivative manufacturers on adverse experience reactions of potential infectious disease transmissions associated with their products.

We believe that these steps are vital additions to our existing efforts to help assure the safety of the blood supply.

As you know, our existing efforts are based on a five-layered system of overlapping safeguards, each layer contributing to blood and plasma derivative safety. These layers are described in detail in my written testimony.

This five-layered system forms a solid basis upon which additional efforts can be built. We are committed to continually addressing all the potential areas of improvement in our blood safety program. Plasma pool size is one such potential issue. Recognizing that plasma pool size is only one of many factors that we are considering in our efforts to minimize the risks associated with the use of plasma derivatives, FDA continues to assess the limits of pool sizes and potential public health benefits.

FDA has brought this issue of plasma pool size before the Blood Products Advisory Committee several times. In March 1995, FDA discussed with the Blood Products Advisory Committee whether reducing the size of plasma pools from which plasma derivatives are

manufactured would be an effective precaution against transfusion transmitted diseases, and under what circumstances FDA should consider mandating limits to the scale which certain plasma-derived products are manufactured. The Blood Products Advisory Committee made no recommendation for upper limits on plasma pool size.

In response to further discussions with consumer groups, and the recommendation of this committee in its August 1996 report, FDA reconsidered the issue of pool size and brought the issue to the December 1996 Blood Products Advisory Committee meeting for reconsideration. At that time, the following limits were constituted for implementation in the short-term: 15,000 donors per pool for products manufactured from source plasma, and 60,000 donors per pool for products manufactured from recovered plasma.

Over the longer term, FDA proposed for discussion further reductions of pool sizes. The Blood Products Advisory Committee determined that data were not sufficient to make a recommendation on upper limits for pool size. The additional data, CBER requested, and continues to request, information from the plasma products industry to better understand the potential public health implications of limiting plasma pool size. CBER recently has received interim responses to its inquiries on plasma pool sizes used by some fractionators in its manufacturing of various plasma derivative products.

This recent information indicates that plasma pool size, after adjustment for combination of intermediates, may result in the pooling of material from several hundred thousand donors for single lots of some products.

FDA does consider there are public health benefits in limiting pool size, particularly for infrequent users of plasma products. The exposure risk for infrequent users would be reduced in instances where the prevalence of the infectious agent is low.

Reduction in pool size also might lessen the impact of recalls and withdrawals on the supply of the products. For the full public health benefit of the smaller pool size to be realized by the recipients of these products, measures also must be taken to ensure that recipients are not simply exposed to more lots of products and thereby essentially the same number of donors.

We have not fully assessed the interim estimates of pool size obtained in response to our inquiries. After more detailed information is collected, analyzed, and verified, we will be able to make a more informed proposal on limiting pool size.

In addition to limiting pool size, we believe there are other approaches to reducing risk, including additional and more sensitive testing methods, improved donor screening procedures, improved viral clearance procedures, and improved plasma management practices. FDA is committed to examining all of these possibilities.

In conclusion, FDA is facing significant changes in helping to ensure the safety of blood and plasma derivatives. We must strive for continued improvements in the regulation and management of plasma derivatives and the plasma fractionation industry. It is important to remember that pool size is only one factor which can be considered in ensuring the safety of plasma derivatives. Good man-

ufacturing practices and our enforcement of those practices is also an important part of the system of overlapping safeguards.

As the Director of the center, I assure you that I am committed to the safety of the blood supply and plasma derivatives. And I will pursue the efforts described with utmost diligence and attention.

Thank you for this opportunity. I'll be glad to answer any questions.

Mr. SHAYS. Thank you.

[The prepared statement of Ms. Zoon follows:]

I. INTRODUCTION

Mr. Chairman and Members of the Committee, I am Dr. Kathryn Zoon, Director, Center for Biologics Evaluation and Research (CBER) of the Food and Drug Administration (FDA). I appreciate this opportunity to discuss the safety implications of plasma pool sizes in the manufacture of fractionated blood products. My testimony also will address FDA measures which ensure the safety of all pooled plasma derived products.

Let me state up front that FDA believes there should be upper limits set on plasma pool size for public health reasons which I will discuss later in this testimony. It is important to remember, however, that pool size is only one factor to be considered in minimizing the risks associated with plasma derived products. This is one of many different approaches to reducing the risks of blood and plasma derived products.

II. PRODUCT SAFETY

On June 5, 1997, FDA testified before this Committee on its efforts designed to ensure the safety of the blood supply, particularly plasma derived products. Let me briefly reiterate some of the most recent steps that FDA has taken directed towards

improving and ensuring the safety of the blood supply and plasma derived products.

CBER's internal emergency response procedures have been improved to facilitate a more effective response to potential and actual emergency situations. The lead responsibility for conducting inspections of plasma fractionators has been transferred to the field organization (Office of Regulatory Affairs). Recent inspections have been more comprehensive and greater attention has been given to good manufacturing practices (GMPs) in the manufacture of blood and plasma derived products. Moreover, FDA has provided enhanced public access to recalls and withdrawals of fractionated plasma products by providing easily accessible information through the Internet, faxes, and e-mail.

FDA continues to utilize a five layer system of overlapping safeguards, each contributing to blood and blood product safety. With donor screening, potential donors are provided educational materials and asked specific questions by trained personnel about their health and medical history. Potential donors whose blood may pose a health hazard are asked to exclude themselves. Donors also are excluded based on risk of malaria, Creutzfeldt-Jakob Disease (CJD), and acute illness. In addition, donated blood is tested for blood-borne agents such as Human Immunodeficiency Virus-1 (HIV-1), HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. Blood establishments must keep current a list of

individuals who have been deferred as blood or plasma donors and check all potential donors against that list to prevent use of units from deferred donors. Blood products are quarantined until products have been tested and donation records have been verified. Blood establishments must investigate any breaches of these safeguards and correct system deficiencies that are found by the establishments or through FDA inspections.

Recent advancements in blood safety include the following. In March 1996, FDA approved the HIV-1 p24 antigen test and issued recommendations for its implementation. These tests improve blood safety by further closing the "window period" before antibodies to HIV develop. FDA issued guidance to blood establishments on the deferral of donors who immigrated from countries with HIV-1 Group O (a new strain of HIV for which testing methods were unavailable). FDA also advised manufacturers of test kits to modify their kits to enhance sensitivity to detect HIV-1 Group O specimens. In September 1996, FDA issued a final regulation on "Current Good Manufacturing Practices for Blood and Blood Components: Notification of Consignees Receiving Blood and Blood Components at Increased Risk for Transmitting HIV Infection." The final rule requires blood establishments and consignees to quarantine previously collected whole blood, blood components, Source Plasma, and source leukocytes from donors with reactive screening tests for HIV. Blood establishments also must perform

confirmatory testing for donations that test reactive for HIV and notify consignees of prior collections so that they may take further action. FDA now requires plasma derivative manufacturers to file monthly reports on adverse experience reactions of potential infectious disease transmissions associated with their products. FDA has been working with manufacturers to ensure that all plasma derived products have adequate viral clearance in manufacturing and, in the interim, has instituted lot release testing for HCV nucleic acid for any non-viral inactivated immune globulins.

III. CREUTZFELDT-JAKOB DISEASE (CJD)

The issues raised in the January 29, 1997 hearing before this Committee on CJD still hold true today. (FDA's January 29, 1997 testimony is appended for reference.) FDA has acted to reduce the theoretical risk of CJD transmission through blood and blood products. Although the risk for transmission of CJD through the blood supply is only theoretical, FDA has acted proactively to defer high risk donors and has recommended voluntary withdrawal of affected products. FDA first issued guidance on CJD in 1987 concerning the deferral of donors who had received human growth hormone derived from human pituitary sources. FDA issued an interim policy in a memorandum dated August 8, 1995, regarding blood products and plasma derivatives. This memorandum further broadened its guidance on donor exclusions for CJD risk and

called for withdrawal of implicated blood products. A provision was made for release of affected products in case of a documented shortage provided that the products carried a special label. In December 1996, FDA issued its latest guidance on precautionary measures to reduce the possible risk of transmission of CJD by blood and blood products. There is presently no test available to screen blood donors for the presence of CJD. In fact, there is still controversy over the nature of the causative agent.

FDA continues to work with it sister agencies, National Institutes for Health (NIH) and the Centers for Disease Control and Prevention (CDC), to review studies and surveillance data on CJD. With CDC and NIH, FDA continues to evaluate the risk of CJD transmission through the blood supply.

IV. BACKGROUND: PLASMA DERIVED PRODUCTS

Each year, approximately 14 million units of whole blood are collected from about 8 million volunteer donors to make components that are transfused directly into more than 3.5 million Americans. Some of the plasma from these collections (recovered plasma from Whole Blood) is used for fractionation into plasma derived products. Approximately an additional 12 million units of Source Plasma are collected through plasmapheresis for processing into derivatives. These products include albumin used to restore plasma volume, clotting factors

used to treat hemophiliacs, and immunoglobulins used to treat or prevent a variety of diseases.

It is estimated that per annum the number of patients who rely on products manufactured from human plasma is as follows: more than 400,000 are given albumin; 15,000-18,000 are given Factor VIII; 3000-5000 receive Factor IX; greater than 20,000 receive intravenous immune globulins (IVIG); and approximately 100,000 to 500,000 receive intramuscular immune globulins (IMIG). Additional patients receive a variety of hyperimmune globulins and other specialized products.

V. PLASMA POOLING AND FRACTIONATION PROCESS

Human plasma proteins for therapeutic use have been manufactured from large pools of plasma for over 50 years. In order to manufacture plasma derived products, most domestic manufacturing facilities have been designed to work at large scales, using large plasma pools to permit manufacturing of sufficient quantities of products. These plasma pools are derived by combining units from individual donations. The number of units combined into a common mixture for processing is known as "pool size." Typically, plasma pool sizes will range from thousands to hundreds of thousands of individual units. For certain products, the use of large pools of plasma (or the pooling of multiple manufacturing batches into larger lots) may contribute to product

consistency and efficacy. For example, the production of Immune Globulin (Human), used to treat Hepatitis A, is mandated by FDA regulation at or above a minimum scale of 1,000 donors to ensure the inclusion of a broad spectrum of antibodies (*see* 21 C.F.R. §640.102(d)).

Units of plasma collected as Source Plasma contain approximately 500-800 milliliters while recovered plasma from Whole Blood donations contain approximately 200-250 milliliters. A pool comprised only of recovered plasma includes units from more individuals donations than a pool of equal volume comprised only of Source Plasma because of the difference in volumes.

The various plasma derived products are purified from the plasma pool by the fractionation process. The basic methods for plasma fractionation were first developed and refined in the 1940s. These methods form the basis for the plasma derivative industry practices today.

Fractionation is a process which separates plasma proteins based on the inherent differences of each protein. Fractionation involves changing the conditions of the pool (e.g., the temperature or the acidity) so that proteins that are normally dissolved in the plasma fluid become insoluble, forming large clumps, called precipitate. The insoluble protein can be collected by spinning the solution at high speeds. One of the

very effective ways for carrying out this process is the addition of alcohol to the plasma pool while simultaneously cooling the pool. For this reason the process is sometimes called cold alcohol fractionation or ethanol fractionation. This procedure is carried out in a series of steps so that a single pool of plasma yields several different protein products, such as albumin and immune globulin.

As knowledge of plasma proteins increased, additional methods were developed to prepare still more unique proteins from plasma. These methods could be added on to the basic cold alcohol fractionation. For example, in the 1960s it was learned that simply thawing frozen plasma at low temperature resulted in a white precipitate called cryoprecipitate that could be separated from the plasma by centrifugation. This substance proved to be very rich in Factor VIII, the clotting factor used to treat Hemophilia A. Factor VIII is then purified from the cryoprecipitate. The plasma fluid left over after the cryoprecipitate is harvested can then be processed to yield albumin and immune globulin.

VI. VIRAL INACTIVATION/REMOVAL PROCESSES

Just as the desired plasma derived products can be separated from each other by chemical or physical means based on the individual properties of the product, contaminating viruses may concentrate

selectively in certain fractions because of their properties. The risk to a patient from any particular agent may vary with the particular plasma derivative. Thus, FDA believes that all plasma derived products should undergo viral inactivation or removal procedures to ensure safety.

Most plasma derivative products are processed to inactivate or remove viruses. At present, the technology exists to inactivate lipid enveloped viruses such as HIV, HBV, and HCV. The technology to inactivate heat stable, non-lipid enveloped viruses, such as the Hepatitis A virus, or agents such as CJD while preserving the functions of plasma proteins is not currently available.

There are highly effective mechanisms for removing or inactivating certain viruses. Two different methods of inactivation are heating and chemical inactivation. These inactivation procedures must be rigorous enough to inactivate the contaminating virus without destroying the plasma derivative. Some manufacturers have incorporated more than one viral inactivation or removal procedure during the manufacturing steps. This combination of inactivation or removal procedures provides additional assurances of safety.

Heat inactivation is the heating of the product at a specific temperature for a specific time under defined conditions. FDA

regulations require that albumin (Human) and Plasma Protein Fraction (PPF) be heated for 10-11 hours at 60 degrees Centigrade in the final container to ensure viral inactivation (*see* 21 C.F.R. §§ 640.91(e), 640.81). Certain viruses, such as HIV, are fairly fragile and are readily inactivated by these heating procedures.

Chemical inactivation involves the addition of certain chemicals to the plasma preparation. For example, some manufacturers add certain solvent/detergent mixtures in their manufacturing processes. The chemicals are removed later in the manufacturing scheme. These processes disrupt viruses that contain lipid-envelopes, such as HIV, HCV, or HBV, without destroying the plasma derived products. HIV and other viruses have a lipid membrane surrounding the viral core. The lipid membrane contains critical viral proteins needed for infection of host cells. Disrupting the viral lipid envelope renders the virus non-infectious. Other viruses which do not have a lipid envelope are not inactivated by these procedures.

Steps that purify the plasma protein may simultaneously remove viral particles whether or not the particles contain a lipid envelope. One example of a viral removal procedure is the use of a monoclonal antibody column to purify a plasma derivative such as Factor VIII. In this instance, antibodies to the Factor VIII are generated in large amounts in tissue culture. The antibodies

are attached to a support within a column. The plasma pool or intermediate product is passed through the column. The Factor VIII binds to the specific antibody while the fluid containing other plasma derived products, and possibly contaminating viruses or other agents, flows through the column. The Factor VIII can later be removed from the antibody column. These processes do not inactivate any contaminating agent but may remove them from the desired Factor.

In the 10 years since the adoption of adequate viral inactivation procedures, there has not been any confirmed case of HIV transmission through a plasma derivative. Recent experience involving Hepatitis A transmission through clotting factors and HCV by intravenous immune globulins produced without adequate viral inactivation procedures, however, reminds us of the need to remain vigilant and to continue our efforts to improve product safety.

Each of these inactivation or removal processes has its particular advantages. Except for the heating of albumin and PPF which is mandated by regulation, the inactivation or removal process may vary with each manufacturer. During the approval process, FDA requires a manufacturer to demonstrate by laboratory studies the effectiveness of its process and to provide validation to ensure that the process works as expected, time after time. FDA evaluates the clearance methods and the

validation studies when reviewing license applications. These operating procedures practices also are reviewed during inspections.

VII. CURRENT PLASMA POOL SIZE ESTIMATES

FDA has not established an upper limit to the size of plasma pools for the manufacture of plasma derived products. FDA regulations are silent on pool size requirements except to specify the minimum pool size for the manufacture of Immune Globulin (Human), a product used to treat Hepatitis A (*see* 21 C.F.R. §640.102(d)). Therefore, FDA has limited information concerning the pool size used by manufacturers of plasma derived products.

According to information obtained from a plasma fractionators' trade organization, for the manufacture of albumin, Factor VIII, Factor IX, and the immune globulins, an initial fractionation pool was estimated to contain 1,000 to 10,000 Source Plasma units, or as many as 60,000 recovered plasma donations. For some products, smaller pools may be used; for instance, specific immune globulins, such as anti-Rho-D[1], are thought to be manufactured from plasma pools collected from between 150 and 2500 donors. Recent information indicates that the pool size,

[1] This is used to prevent complications during pregnancy when there are different Rh types between the mother and fetus.

after adjustment for combination of intermediates, may result in the pooling of material from several hundred thousand donors for single lots of some products.

In some cases, the plasma pool size equals the sum of all plasma donor units used in the starting pool for manufacture of one lot of final product. More often during manufacturing, intermediate material derived from more than one starting pool may be combined into one lot prior to processing into final product. In these cases, the plasma pool size is the sum of all the plasma pools from which the intermediate products were derived. Plasma derived products from other pools may be combined during the fractionation process or added to the final product. For example, albumin is added during intermediate processing steps or to a final product, such as Factor VIII, for use as an excipient or stabilizer. This albumin often has been derived from another plasma pool.

VIII. CURRENT FDA ASSESSMENT OF POOL SIZE

FDA's goal is to continue to minimize the risks associated with blood and plasma derived products. One possible means of minimizing risk is to reduce plasma pool size in the manufacturing of plasma derived products. FDA believes that there should be upper limits set on plasma pool size for public health reasons.

The benefits of limiting pool size are that the infectious risk for infrequent users would be reduced in instances where the prevalence of the infectious agent is low. Reduction in pool size might also lessen the impact of recalls and withdrawals on supply of the products.

In setting upper limits on pool size, potential adverse consequences also must be considered. Decreasing pool size may decrease the number of vials available from a batch. With small size batches, quality monitoring and release testing could consume a large portion of the batch. Decreasing batch size in existing plants may result in sub-optimal processing. Decreasing batch size in existing plants might decrease overall product availability.

It should be noted, also, that reducing pool size necessarily would require the production of a larger number of lots of any given product to be produced in order to maintain the supply of that product at a constant level. Therefore, for the full benefit of the smaller pools to be realized by the recipients of these products, measures also must be taken to insure that the recipients are not exposed to more lots of product and, thereby, to more pools.

It may be that there are other approaches to reduce risk, including additional and more sensitive testing methods, improved

donor screening processes, improved viral clearance procedures, and improved plasma management practices. FDA is committed to examining all of these possibilities

IX. HISTORY OF FDA ACTIONS RELATING TO POOL SIZE

Many of the steps mentioned above, such as donor screening, donor testing, donor deferral, product quarantine, reporting requirements, viral clearance, and FDA inspections, greatly reduce the disease risks associated with plasma derived products. Continuous efforts, however, need to be made to reduce the risks to ever lower levels. Although reducing the pool size of plasma derived products has been under consideration for some time, increased attention to this issue has risen because of consumer interest, CJD, and recent recalls.

At the March 1995 Blood Products Advisory Committee (BPAC) meeting, FDA asked whether reducing the size of plasma pools from which plasma derived products are manufactured would be an effective precaution against transfusion-transmitted diseases and under what circumstances FDA should consider mandating limits to the scale at which certain plasma derived products are manufactured. BPAC made no recommendation to adjust pool size for plasma derived products.

In response to further discussions with consumer groups and recommendations of this Committee in its August 1996 report, FDA reconsidered the issue of pool size and brought the issue to the December 1996 BPAC for reconsideration. The following limits were discussed for implementation in the short term: 15,000 donors per pool for products manufactured from source plasma and 60,000 donors per pool for products manufactured from recovered plasma. Over the longer term, FDA proposed, for discussion, further reductions of pool sizes. Additionally, FDA also proposed that whenever possible a plasma derivative such as albumin that is added as a stabilizer or excipient to another product should be derived from the same plasma pool as the product to which it is added.

Industry representatives voiced concerns that significant reductions in plasma pool size were not feasible primarily due to cost and manufacturing capability considerations. No recommendation on pool size was made by BPAC as it was determined that there was insufficient data on which to base a policy decision.

On February 7, 1997, the International Plasma Products Industry Association (IPPIA) wrote FDA after reviewing the proposals made at the December 1996 BPAC meeting and urged that there be no further discussion on limiting pool size. The letter stated that no safety benefits would result from pool size reduction.

Further, IPPIA stated that pool size reduction would result in significant product supply reductions, as well as very significant time and costs increases involved for remodeling manufacturing facilities to accommodate smaller production scale equipment.

In response to that letter, FDA stated a continued interest in setting practical upper limits on plasma pool size. FDA pointed out that pool size limits could limit risk of disease transmission for patients who are infused infrequently and lessen the impact of product withdrawals or recalls. This remains FDA's position.

On June 26, 1997, FDA sent a letter to certain plasma fractionators requesting detailed information on plasma pool size. The purpose was to collect additional information upon which to base a final decision on pool size. The letter also asked for information on plasma recovery practices which may further increase the pool size. FDA has not fully assessed the interim estimates of pool size obtained in response to this letter. After more detailed information is collected, analyzed, and verified, FDA will be able to make a more informed proposal on limiting pool size.

Since FDA's request, IPPIA has expressed interest in continuing to discuss with FDA the issues related to increased plasma

product safety. IPPIA met with FDA on July 14 to further those discussions.

X. CONCLUSION

FDA faces significant challenges in helping to ensure the safety of plasma derived products. We must strive for improvements in the regulation and management of plasma derived products and the plasma fractionation industry. As a part of this effort, we believe that there should be practical upper limits on pool size for the above-mentioned public health reasons. It is important to remember that pool size is only one factor to be considered in ensuring the safety of plasma derived products. Good manufacturing practices and our enforcement of those practices is also an important part of the system of overlapping safeguards that contribute to the safety of plasma derived products, and we remain vigilant in our efforts to enforce the good manufacturing practice regulations through the use of regulatory inspections.

Mr. Chairman, Members of the Committee, thank you for the opportunity to participate in today's hearing on the proposed rule on the ruminant to ruminant feed ban and the potential transmission of spongiform encephalopathies to humans. My name is Dr. Michael Friedman and I am the Deputy Commissioner for Operations at the Food and Drug Administration (FDA).

FDA is the nation's oldest consumer protection agency, responsible for the safety and effectiveness of over $1 trillion worth of products and commodities. We have been protecting consumers against an ever-growing number of public health risks for over nine decades, and we have not done it by standing still. FDA constantly is being presented with new questions, for which we are committed to seeking and finding answers, while applying current statutes, state of the art science, and knowledge gained from our experiences in responding to previous public health risks.

FDA's responsibilities encompass drugs for use in people and animals, human biological products, medical devices, food, dietary supplements, cosmetics, and animal feeds. Each of these product groups has been considered with respect to the potential for the transmissible spongiform encephalopathies (TSEs) in humans or animals. As you may know, TSEs are a group of transmissible, slowly progressive, degenerative diseases of the

central nervous systems of humans and several species of animals. This family of diseases is characterized by a long incubation period, a relatively short clinical course of neurological signs, and 100 percent mortality. Examples of TSEs are scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, transmissible mink encephalopathy, chronic wasting disease of deer and elk, and Creutzfeldt-Jakob Disease (CJD) and kuru in humans.

FDA has been involved actively in national and international efforts focused on better understanding TSEs. FDA has collaborated extensively with its sister Public Health Service Agencies, the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), and with the United States Department of Agriculture (USDA), as well as with affected industries and consumer groups. FDA has formed an intra-agency working group composed of myself and experts from each FDA Center to consider transmissible spongiform encephalopathies and their impact on FDA-regulated products. A CJD Advisory Committee, composed of outside experts, including academic and government representatives; consumer groups, including the National Hemophiliac Foundation; and industry groups, also was formed in 1995, and was rechartered in June 1996 for two additional years as the TSE Advisory Committee.

I. BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Bovine spongiform encephalopathy (BSE), also referred to as "mad cow disease," is thought to be a transmissible, slowly progressive, degenerative disease of the central nervous system of cattle, and is similar to scrapie in sheep. BSE has a prolonged incubation period in cattle (three to eight years) following oral exposure and, as with all TSEs, once symptoms appear, BSE invariably is fatal. There is no known treatment or cure. That said, we emphasize that BSE has not been detected in cattle in the U.S., and since 1989, no cattle have been imported from BSE countries as designated by the USDA.

Since BSE was first diagnosed in the United Kingdom (UK) in November 1986, more than 165,000 cattle from almost 33,000 herds have been diagnosed with the disease. BSE now has been reported in native cattle in France, Switzerland, Portugal, the Republic of Ireland, and Northern Ireland. The epidemic in the UK peaked in January 1993 at nearly 1,000 new cases per week; currently fewer than 200 suspected cases are diagnosed every week. The disease has had a devastating impact on the cattle and beef industry in the UK where hundreds of thousands of suspect cattle have been killed and incinerated to prevent further spread of the disease.

Epidemiological studies, including computer simulation of the epidemic in the UK, suggest that feeding cattle rendered meat and bone meal from animals infected with some TSE agent was the vehicle for the spread of the disease. The practice of adding meat and bone meal to animal feed has become a common way for producers to supplement their animals' protein and other dietary needs. Possible hypotheses as to the original UK source of the TSE agent are: 1) that it was a modified form of scrapie transmitted via rendered by-products of sheep, or 2) that it was a cattle-adapted strain of a scrapie-like agent, also spread via feed. Both theories are consistent with the epidemiological findings. Of particular importance, recent research in the UK suggests that the BSE agent is resistant to the rendering processes used in that country. This research further supports the epidemiological evidence that the disease has been spread through rendered meat and bone meal added to cattle feed. Scientists also have theorized, however, that BSE could occur spontaneously in cattle, though presumably at a very low rate.

Possible Link Between BSE and CJD

Scientists also have theorized about the impact of BSE on human health and its possible link to CJD. CJD is a slow degenerative human disease of the central nervous system characterized by motor dysfunction, progressive dementia, and vacuolar

degeneration of the brain. The incidence of CJD in the U.S. is similar to the incidence in the rest of the world. Sporadic cases of CJD occur world-wide at a rate of one case per million population per year.

On March 20, 1996, the British Government announced a possible link between BSE and ten cases of a new type of CJD in humans. These recent cases appear to represent a new variant of CJD (nv-CJD) that seems to be unique. At a World Health Organization (WHO) consultation in April 1996, a group of international experts concluded that there is no definite link between BSE and this small group of people with nv-CJD, but epidemiological evidence suggests exposure to BSE before the UK specified tissue ban in 1989 may be the most likely explanation. To date, scientists have identified the distinctive nv-CJD brain pathology in 15 people with CJD in BSE countries (14 in the UK, 1 in France). In October 1996, Dr. John Collinge, one of the foremost British authorities on CJD, and his colleagues published the results of their research on various strains of the agents believed to transmit BSE. The results suggest that the agent found in nv-CJD resembles the BSE agent rather than the sporadic CJD agent.

As stated, BSE has not been detected in cattle in the U.S., and since 1989, no cattle have been imported from BSE countries as

designated by USDA. Nevertheless, the possible link between nv-CJD and BSE, and new information about the origin and etiology of the BSE agent have prompted the public, the U.S. Government and affected industries to view this disease very seriously. As a result, several important measures have been undertaken to further reduce the remote risk of BSE occurring in the U.S. It should be stressed, however, that there is no established scientific link between BSE and CJD in humans.

Proposed Rule: Ruminant and Mink to Ruminant Feed Ban

One critical measure is the issuance by FDA of a Notice of Proposed Rule Making (NPRM) to prohibit the use of nearly all tissues from ruminants -- animals such as cows, sheep, and goats -- in feed intended for ruminants. Mink tissue also would be prohibited from such feeds, because of known cases of TSE in mink raised in the U.S. The prohibition on feed ingredients proposed in the NPRM is intended to prevent the spread of BSE in cattle in the unlikely event that the disease should ever occur in this country, and to further minimize any risk that might be posed to humans. The NPRM was published in the Federal Register on January 3, 1997, after FDA completed an in-depth review of the 660 comments it received last year in response to its advance notice of proposed rulemaking related to the feed ban. These comments covered many of the scientific and economic issues

addressed in the NPRM. FDA's proposal to ban the use ruminant and mink proteins in ruminant feed follows a voluntary industry moratorium on similar feeding practices instituted in March 1996 by national livestock organizations and professional animal health groups and endorsed by FDA, CDC, NIH, and the USDA. The finalization of the proposal will add another level of safeguards to protect the U.S. against the remote potential risk from TSEs. Moreover, FDA's proposed regulation is supported by last year's WHO recommendations for countries in which no BSE has been diagnosed.

The NPRM provides that animal protein derived from ruminant and mink tissues are not generally recognized as safe (GRAS) for use in ruminant feed and is a food additive subject to section 409 of the Federal Food, Drug, and Cosmetic (FDC) Act. The determination of food additive status for this substance (protein derived from ruminant and mink tissues) will help to ensure that it will not be marketed in the U.S. until such time as FDA determines it to be safe. The NPRM proposes to exempt from the ruminant protein feed ban three tissue types that have shown no signs of potential infectivity. These exceptions include bovine blood, ruminant-derived milk, and gelatin. A second component of the rule provides for a system of processes and controls, including record keeping and labeling, that is necessary to ensure the proposed rule will achieve its intended purpose.

Based on the overwhelming evidence we have on transmissibility, if for some reason a case of BSE were to occur in the U.S., and it is important to reemphasize that not even one case of BSE has ever been found here, the steps being proposed in the NPRM would confine it to the individual animal and greatly decrease the potential risk to other animals and humans.

The preamble to FDA's proposed rule points out that FDA is considering alternatives to the proposed ruminant and mink protein to ruminant prohibition and that it also is seeking comments on those alternatives, which include a(n): (1) Adult sheep and goat specified offal to ruminant prohibition; (2) Prohibition to ruminants of all materials from U.S. species which have been diagnosed with TSEs (sheep, goats, mink, deer, and elk); (3) Partial ruminant to ruminant prohibition; (4) Mammal to ruminant prohibition; (5) No regulatory action; and, (6) Other alternative approaches that meet FDA's regulatory objective.

Although BSE does not exist in the U.S., we believe that the preventive approach FDA is taking in the NPRM is justified by what we now know about this disease and how it is caused and spread. As noted above, epidemiological evidence corroborates the theories that the origin of BSE was caused by the feeding to cattle of meat and bone meal either containing the scrapie agent

from rendered by-products of sheep, or a cattle-adapted strain of a scrapie-like agent from rendered by-products of cattle. Current U.S. rendering techniques would decrease, but probably not totally eliminate the BSE agent. Since sheep scrapie and other animal-borne TSEs already are known to exist in the U.S., the epidemiological evidence indicates that BSE could possibly develop and be spread here under unrestricted feeding practices. Moreover, the risk that BSE-infected cattle or feed could be imported inadvertently from BSE infected countries, or that BSE could occur spontaneously further supports the preventive strategy proposed in the NPRM. The strategy provided in the NPRM also is supported by the steady decline in the number of cases of BSE in the UK after they established similar restrictions on ruminant feeding practices.

Comments are being solicited by FDA on all aspects of the NPRM, including the scope of the ban and the list of exempted tissue types. A 45 day public comment period expires on February 18, 1997. To facilitate notice and comment on the NPRM, in addition to providing the proposed rule to the CDC, NIH and the USDA, FDA provided a copy of the proposed rule to the group of international experts interested in BSE. The document was delivered to heads of foreign public and animal health organizations and to appropriate officials of our major animal products trading countries. During February, FDA will hold two

open public forums to discuss the notice of proposed rule making to prohibit the use of rendered ruminant and mink protein products in ruminant feeds. Comments may be submitted to the Dockets Management Branch, Food and Drug Administration, 12320 Parklawn Drive, Room 1-23, Rockville MD 20857. These comments will be reviewed by the Food and Drug Administration and will be used in preparing final regulations.

FDA recognizes that American consumers look to us to assure the safety of the U.S. food supply. We believe that the strong preventive strategy provided in the NPRM is supported by the best available science on BSE and that this approach significantly reduces risk to animal health and any perceived risk to human health. As the scientific knowledge about BSE, and all TSEs, increases -- and the science in this area is growing rapidly -- FDA will continue to review this new evidence and steer a course that maintains high standards for food safety in this country.

II. CJD AND PROTECTION OF THE BLOOD SUPPLY

The history of TSE raises questions regarding the transmissibility of CJD through human tissue. While there are no recorded cases of CJD transmission in humans through blood, there

is a theoretical possibility for transmission and FDA has taken aggressive actions to significantly mitigate that potential risk.

The blood supply plays a critical role in the American health care system. While the U.S. has one of the safest blood supplies in the world, it is a formidable task to keep it so. Each year, approximately 12 million units of blood are drawn from volunteer donors for use by more than 3.5 million Americans. Much of this blood, and an additional 12 million units of plasma, is processed into further products, referred to as derivatives, such as immune globulin, used to prevent infections, and clotting factors, such as antihemophilic factor, used to treat bleeding disorders.

Because blood donors may harbor undetectable or undetected communicable disease, blood can transmit disease. Because of this risk, and the fact that millions of Americans depend on blood and blood products, efforts to ensure the safety of the blood supply are a high priority for FDA. One of the challenges such efforts entails is application of current, but incomplete and emerging scientific knowledge, in the decisions about how best to protect public health. CJD presents such challenges.

Background/History of CJD

CJD is a rare but invariably fatal, degenerative neurological disease believed to be associated with a transmissible agent. Cases arise spontaneously at low frequency for unknown reasons; perhaps acquired by external exposure to infectious material; or may arise spontaneously at higher frequency in persons with certain genetic mutations. CJD affects approximately one person to two person per million per year worldwide. From 1979 through 1994, CJD was recorded as a cause of death in 3,642 deaths in the U.S.; representing a stable incidence. (CDC Dispatches, *Emerging Infectious Diseases*, Vol. 2, No. 4, October-December 1996). The clinical latency of CJD can exceed thirty years, although the incubation period is shorter for the known iatrogenic cases.

The nature of the transmissible agent for CJD is not established, but seems to be highly resistant to the current methods of viral inactivation employed with plasma derivatives. The disposition of the agent during fractionation of various plasma derivatives is not presently known.

Between 1983 and 1997, approximately 300 million units of blood and plasma were donated. From 34 reports received (with some reports containing information on more than one donor), 37 donors were either subsequently diagnosed with CJD or deferred based on

concerns with CJD. Of the 37 blood/plasma donors, 25 were reported as subsequently diagnosed with CJD; four had a family member who was diagnosed with CJD; four had received Human Pituitary-Derived Growth Hormone (HGH); and, four had received a dura matter transplant.

The available basic scientific and applied epidemiological data provide no evidence of transmission of CJD via blood transfusion in humans. Moreover, transmission by intravenous infusion of whole blood from CJD patients has not been demonstrated in subhuman primates. The disease, however, has been verified to be transmitted between humans by transplantation of corneas and cadaver dura mater grafts from affected individuals, by use of contaminated EEG electrodes, by certain neurological procedures and by injections of HGH. (CDC Dispatches, *Emerging Infectious Diseases*, Vol. 2, No. 4, October-December 1996). (It should be noted that HGH is no longer used having been replaced by a recombinant-DNA derived alternative product.) In addition, the disease has been transmitted to rodents in laboratory experiments by injecting the buffy coat component of blood from an affected patient into the rodent brain. Although CJD has occurred in transfused patients, we stress that there has <u>not</u> been a documented case of CJD being transmitted through a blood

transfusion. Moreover, there has not been an identified case of hemophiliac death from CJD.

We know that despite our best efforts blood and blood products will never be totally risk free, but we continue to work to achieve optimal safety and availability. While our current knowledge of blood-borne diseases has improved significantly over the past 10-15 years, current scientific knowledge is still incomplete. For example, there is currently no serum test capable of detecting CJD infection.

The fact that there are no documented cases of blood or blood product transmission of CJD in humans does not end the inquiries into the disease nor does it mean that FDA and other agencies and research entities can be complacent. Basic and applied research into the infectious processes of CJD continue to serve as the catalyst for the evolution of FDA policy. We cannot let the absence of scientific information paralyze us.

FDA Regulatory Response

The development of FDA regulatory policy with respect to blood products that could possibly carry a risk of the disease CJD has been vigorous and is ongoing. As clinical and epidemiological knowledge of CJD has increased, FDA has responded aggressively by

reviewing and modifying its policy. Throughout this process, FDA has worked closely with both CDC and NIH, among others, in determining the most appropriate regulatory course of action. Extensive public discussion with all segments of those affected - recipients of products, medical professionals, academicians and industry -- has been conducted throughout FDA deliberations.

FDA Actions Related to Blood and Blood Products

FDA involvement in addressing the possible impact of CJD on the nations's blood supply began with the early awareness of possible transmission. On November 25, 1987, FDA issued a "Memorandum to All Blood Establishments" entitled "Deferral of Donors Who Have Received Human Pituitary-Derived Growth Hormone." This document recommended that all persons who received HGH be barred permanently from donating blood or plasma.

For the period 1983-1992, there were only four reported blood donors who had a confirmed diagnosis of CJD reported post-donation. In response, the blood and plasma product manufacturers initiated a voluntary withdrawal of in-date products that had been prepared from donations from these individuals. In December 1993 FDA expanded its position and issued recommendations for more complete reporting of "post donation information" related to safety.

Partly as a result of these FDA policy recommendations, in late 1994 and early 1995 FDA began receiving additional reports of CJD affected individuals who had donated blood and plasma. At FDA's request, the manufacturers placed in-date, licensed, injectable derivatives (of blood and plasma), as well as intermediates (those products used in further processing), into quarantine awaiting development of FDA recommendations on the use of implicated material.

At the Blood Products Advisory Committee (BPAC) FDA presented data regarding the biology of CJD and case histories of CJD-related donor deferrals and product withdrawals on December 15, 1994. In March 1995, BPAC was again updated on CJD. BPAC was presented with the available scientific information and options for action. BPAC was unable to reach consensus decisions on all of the issues related to product disposition and recipient notification.

FDA, in an effort to further develop its policy on CJD, and because of the outstanding issues that required additional public discussion and consideration, formed a Special Advisory Committee on Creutzfeldt-Jakob Disease and presented information to it on June 22, 1995. The Special Advisory Committee agreed that:

- there was no scientific evidence that CJD is transmitted by transfusion of blood products or by administration of plasma

derivatives;

- implicated blood components should be withdrawn;
- implicated plasma derivatives should be withdrawn; and
- if implicated blood components and/or plasma derivatives are to be released for transfusion, these products should bear special warning labels.

Within two months, after considering these deliberations and extensive internal discussion, FDA issued an interim policy in a memorandum dated August 8, 1995, regarding blood products and plasma derivatives. This further broadened its guidance on donor exclusions for CJD risk and called for withdrawal of implicated blood products. A provision was made for release of affected products in case of a documented shortage provided that the products carried a special label.

In an effort to further expand the knowledge of CJD, FDA and NIH's National Heart, Lung, and Blood Institute held a CJD Workshop on Design of Experimental Studies of Transmission of CJD. The FDA also held many discussions at BPAC on product warning labels.

To avoid the disposal of safe and effective products while protecting public health and safety, FDA consulted extensively with experts in the field of TSEs on the familial nature of some

CJD cases and appropriate use of genetic testing to clarify risk. The Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) (formally known as the Special Advisory Committee on Creutzfeldt-Jakob Disease) met a second time on July 2, 1996, and discussed refinements to the August 1995 policy. These included the option of reentering deferred donors based on genetic testing results and the disposition of plasma derivatives prepared from product collected from donors with only a single family member diagnosed with CJD. Also, in November 1996, FDA, in cooperation with others, held two public meetings/workshops. Notification procedures to be utilized for implicated products were considered.

FDA revised its recommendations for CJD in a memorandum to manufacturers on December 11, 1996, based on opinions of the advisory committees, public discussion, FDA internal deliberations and discussions with other agencies. The December 11 memorandum updated and superseded the FDA memoranda of August 8, 1995 and November 25, 1987.

FDA, in its December 11 Memorandum, reiterated that the assessment of CJD risk in the donor is a critical responsibility of the blood establishments. In particular, FDA emphasized that family history of CJD requires careful investigation. FDA has

recommended that a family history of CJD should be confirmed by a physician and documented on the basis of currently accepted diagnostic procedures. Also, familial risk, in the context of a donor, applies only to blood relatives of non-iatrogenic cases.

The cautious approach the FDA has taken on CJD related to blood products also affects other products. There are estimated to be over 500 products which use plasma products/derivatives either in manufacturing or formulation. Plasma for manufacturing comes from approximately 25 million donations of blood and plasma derived from about 10 million blood and plasma donors per year. Over a ten year period, 1984-1993, plasma fractionation capacity worldwide increased 61 percent. (Robert, *Journal of the American Blood Resources Association*, at 75, Vol. 4, No. 3 1995).

There is ongoing discussion being conducted by FDA and others concerning the level of risk of CJD transmission in plasma derivatives and blood components. Experiments to quantify this risk are being undertaken by the government and the blood industry. However, it is likely to take several years before conclusive results are obtained. Most scientists believe that any risk from plasma derivatives must be significantly less than from whole blood components.

Despite this inability to more precisely quantify the nature of the risk, as a precaution, FDA has recommended that Source Plasma and plasma derivatives, prepared from donors who are at increased risk for developing CJD, should be quarantined and destroyed. FDA has made an exception from this quarantine for the plasma derivatives, i.e., albumin, immune globulin, etc. (licensed, injectable products), prepared from pools which contain products collected from a donor with only one known family member with CJD. This exception is made because the probability that the case represents familial CJD is low.

FDA has not recommended the quarantine of blood products intended for further research or manufacturing into non-injectable products. FDA has recommended, however, that such products should be labeled with the following statements: 1) "Biohazard"; 2) "Collected from a donor determined to be at risk for CJD"; and, 3) "For laboratory research use only", or "Intended only for further manufacture into non-injectable products".

In the circumstances of a donor with CJD or at increased risk for CJD, consignee notification is recommended to permit recipient tracing and notification as deemed medically appropriate. Given the limited current knowledge about CJD as it relates to blood safety, FDA has made only a few general

recommendations about "lookback" notification. In the event that a donor gives a history of only one known family member with CJD, FDA does not recommend notification of consignees of plasma derivatives or expired blood components.

FDA policy on protection of the blood supply from the remote possibility of CJD transmission has been developed as knowledge and data has evolved. The recommendations for donor deferral, product disposition, and recipient notification have been developed based on a consideration of risk in the donor, risk from the product, and the potential impact on blood product availability. Given the significant size of the population using these products, it is appropriate for FDA to consider the impact of withdrawal from distribution of plasma products in its risk benefit analysis from the perspective of both the effect on the supply of products and the benefit to potential recipients of the products. In its decisions, FDA attempts to balance the need for the products and the risk of using the products.

FDA Decision Making: Case Study

The application of FDA policy in situations involving CJD is evidenced by a recent case. FDA was notified that the certain lots of anti-hemophilic products were manufactured using an ingredient, which had been prepared from pooled plasma,

containing a unit from a blood donor who later died of CJD. These particular anti-hemophilic products had not been released (for distribution) nor approved for release by FDA at the time of the notification to FDA.

In this case, FDA was faced with evaluating the potential risk of CJD in the final products; determining whether FDA should approve release of the lots; and, if released was approved, should any type of notification be provided.

FDA requested a risk assessment from the company which concluded that the risk of CJD in the product was negligible. This risk assessment was reviewed internally and independent risk assessments were obtained from CDC, NIH and Johns Hopkins University. All agreed that the risk, if any, was likely to be remote and considered "vanishingly small" in one analysis. An assessment also was made of the impact on the supply of purified factor VIII available for recipients if the product was not released. The conclusion, based on the scientific analysis and all available, relevant data, was that there was a remote risk of CJD and the products were suitable for release. FDA requested, however, that the situation be conveyed to the affected community -- the hemophilia organizations. The company informed the hemophilia groups. The groups released information on the situation in a community newsletter and other sources.

FDA continues to develop its policy and evaluate the safety of products that have had exposure to an implicated plasma derivative (usually transferrin or albumin) during manufacturing or formulation as new data and information are available.

Adequacy of FDA Response

Although FDA's regulatory response to CJD was initiated long before 1995, the recommendations and comments of the Institute of Medicine (IOM) in its 1995 report could be considered as a framework for evaluating FDA's actions concerning the possible CJD transmission in the blood supply. In its 1995 report *HIV and the Blood Supply: An Analysis of Crisis Decisionmaking*, the IOM concluded that FDA had "missed opportunities" for action in addressing the potential for HIV infection in the blood supply and had chosen "the least aggressive option that was justifiable." The report acknowledged that previous decisions were made "in the context of great uncertainty" given the science. When "knowledge is imprecise and incomplete," however, IOM recommended that there should be "a more systematic approach to blood safety regulation when their [sic] is uncertainty and danger to the public."

The IOM made several recommendations directed specifically at FDA which mirror FDA's actions in developing responses to possible CJD transmission through the blood supply.

Recommendation 6 of the IOM report stated:

> Where uncertainties or countervailing public health concerns preclude completely eliminating potential risks, the FDA should encourage, and where necessary require, the blood industry to implement partial solutions that have little risk of causing harm.

Recommendation 7 of the IOM report stated:

> The FDA should periodically review important decisions that it made when it was uncertain about the value of key decision variables.

FDA has undertaken to incorporate these recommendations into its decision making and oversight of the nation's blood supply. The discussion of FDA's actions taken in response to the concerns raised by possible CJD transmission illustrate that FDA has benefited from past lessons and has responded to the challenge of dealing with uncertain risks that could impact the safety and availability of blood and plasma products.

This Committee also made recommendations in its report *Protecting the Nation's Blood Supply From Infectious Agents: The Need For New Standards To Meet New Threats* (House Report 104-746, August 2, 1996). In response to the Committee's concerns, FDA has provided enhanced public access concerning recalls and withdrawals of blood and blood products; increasing public input in the discussion regarding policy development on withdrawals and notification of plasma products; and, continuing research into the risk factors associated with pool size of donors.

FDA has made information concerning recalls and withdrawals widely available to interested and affected parties. A voice information system with toll free lines has been set up with information on fractionated product recall and market withdrawal information. A fax information system has been put into place allowing "fax-on-demand." The FDA Home Page contains the recall and withdrawal information and an automated e-mail system has been established to distribute information to those persons desiring information not only on recalls and withdrawals but all blood related public documents.

CONCLUSION

FDA continually strives to make the food and blood supply safer. We will continue to evaluate new studies, scientific and epidemiological data on TSEs and apply that knowledge to FDA policy. We look forward to working with the Committee on these issues.

Mr. SHAYS. Why don't we start in—and I'll start with you, Dr. Zoon. What is the largest pool size that has been reported to the FDA?

Ms. ZOON. The interim information that we have received to date, approximately 400,000, if one includes the pooling of intermediates.

Mr. SHAYS. And was that a surprise to you, this size?

Ms. ZOON. I would say that that was larger than I had anticipated.

Mr. SHAYS. What would explain why the FDA wasn't able to tell us the pool size? Is it just something you hadn't focused in on or——

Ms. ZOON. We had information that is available to us from a variety of sources. One initially was some information that we had received from ABRA, which is the association—let me see if I can remember—the Association of Blood Resource—American Blood Resources Association. And those estimates that we were given at that time were approximately 10,000, I believe.

However, further information upon receipt of the request FDA issued to nine of the major plasma pools, there is clarification also at the BPAC advisory committee that, in fact, these reflect the primary pool sizes and did not include estimates of the intermediate pooling or consideration of adding excipients to the purified or the final product.

Mr. SHAYS. Is it fair to say the FDA was thinking that these pool sizes were more like 10,000 and then learned it was 60,000? But wouldn't it be pretty surprising for you all to have learned that it was 400,000 in one instance? I mean, was that a surprise?

Ms. ZOON. I think the number of 400,000 was high. I think at the Blood Products Advisory Committee earlier, I believe a presentation was made by one of the blood associations, that it was potentially as high as 100,000. But 400,000, I think was higher than I would have predicted.

Mr. SHAYS. Does that give the FDA a greater interest in trying to take a look at this issue?

Ms. ZOON. Well, we are committed to putting a limit on pool size.

Mr. SHAYS. OK.

Ms. ZOON. And I think as we get additional information and analyze it and verify that information, we will certainly view limits on pool size as part of a—our recommendations.

Mr. SHAYS. OK.

Dr. Satcher, I got the sense from you that you were basically saying we needed to obviously be pretty cautious when we get into this area for a variety of reasons. And Dr. Brown, from your comments, I made an assumption that one individual could contaminate the whole lot. And the whole pool. But from your testimony, it made me wonder if you were saying to us that a large pool could make the one bad donor almost insignificant because it would be spread out over so many, I just want to clarify that, without it being diluted.

Dr. BROWN. The significance, in my judgment, would be the same. And the concept of a fully dispersed small number of particles——

Mr. SHAYS. Right.

Dr. BROWN [continuing]. Is crucial here. Already at the smallest pool size that's made, probably the number of infectious particles are already fully dispersed and they're in their 40 doses whether it's 100,000 doses or——

Mr. SHAYS. I need to understand you in my way of thinking.

Dr. BROWN. OK.

Mr. SHAYS. The question I am asking, you are answering it, but I am not hearing you right, so let me just say it again, and maybe you can put it in my terms. I just want to be clear. Can one donor in a very large pool be so diluted that it doesn't have significance? Or will there be—will some of the pool be polluted, will be contaminated, or will the whole pool be contaminated with one bad donor?

Dr. BROWN. Some of the pool.

Mr. SHAYS. OK.

Dr. BROWN. And it doesn't much matter whether it is 10,000, 100,000, or a million. The same amount, the same number of donors will be at risk—excuse me, the same number of recipients will be at risk.

Mr. SHAYS. Right. But some will not actually end up with contamination.

Dr. BROWN. That's correct. If you have 10,000 doses and say 5 infectious particles, 5 people are going to be at risk and the rest of them will not be.

Mr. SHAYS. I got you.

Dr. BROWN. Yes.

Mr. SHAYS. It would seem to me, maybe I guess we will get into the whole economies of it, but it would seem to me that using—let me back up and say does that only relate to CJD or does it relate to all types of contamination?

Dr. BROWN. It may relate to more than CJD if we're talking about unknown agents. It certainly, I think, relates to CJD in a way that it does not relate to things like hepatitis and HIV, correct.

Mr. SHAYS. So with HIV, if a large pool is contaminated, that entire plasma will be contaminated, or just again particles hit or miss?

Dr. BROWN. My understanding is, using this analogy of a large number of particles versus a small number of particles, a much greater amount of infectivity will be distributed. And many more individuals would be infected than is true for CJD, which, although we haven't measured it in humans, we have a pretty good idea from experiments that the amount of infectivity, even in an infected animal or human, is very, very small.

Mr. SHAYS. OK.

Dr. Satcher, do you want to respond to any of the questions I asked?

Dr. SATCHER. No, except to restate the fact that we support FDA's commitment to reexamine this issue and to take advantage of the benefits of smaller pool sizes. We realize that there are some other issues involved, like the pool size required for immunoglobins, for example, that we need in these pools. And also, the whole issue of the supply and the effect of pool size on the supply of available plasma products. But given that, yes, we support the direction of FDA.

Mr. SHAYS. OK. Before this panel leaves, I am going to want us to just list the advantages of a large pool size and the disadvantages. But, I would like to move to Mr. Towns.

Mr. TOWNS. Thank you very much, Mr. Chairman. So could I just sort of get the format, are you going to have a second round or 5 minutes, Mr. Chairman?

Mr. SHAYS. Pardon me?

Mr. TOWNS. Five minutes or a second round?

Mr. SHAYS. No, no. You have as much as you want. You just move along.

Mr. TOWNS. OK. Thank you very much.

Dr. Brown, you have conducted experiments on rodents and CJD. First of all, have those studies had peer review?

Dr. BROWN. Yes, they have from one journal.

Mr. TOWNS. And what do you believe the results mean for humans?

Dr. BROWN. I think they put us on an alert status, which is to say, granted we can't infer from rodent studies what exactly is happening in humans, but, as you know, we don't have 500 disposable humans to experiment on, so rodents and primates and experimental animals are the only way to go.

I think what we have shown is that not only is there potentially infectivity present in blood as a whole, but we have defined where in the blood we have to be most careful. And they include at least two plasma fractions. Therapeutic products are made from plasma. And plasma is processed and then made into products such as antihemophiliac factor and immunoglobulin.

The first step in that is a step called Cohn fractionation. The plasma is made into fractions and each one of those fractions is a source of a specific therapeutic product. We've determined that, at least in the rodent experiments, and using inoculation of specimens directly into the brain, again not the same thing as transfusing an animal, that infectivity can be detected in white cells, in plasma, in cryoprecipitate, which is the source of Factor VIII, and in what is called Cohn fraction 1, plus 2, plus 3, which is the source of immunoglobulins. We have not detected infectivity in the two last Cohn fractions, which among other things is the source of albumin.

Mr. TOWNS. Thank you.

Dr. Satcher, can you tell me about the CDC's efforts to establish active surveillance systems in six States and tell us what we can, in Congress, can do to help you establish the similar surveillance systems in the other 50 States? And also name the States you have surveillance in. I know Connecticut is one.

Dr. SATCHER. You—I'm glad you said it. Now I know what you're talking about. You're talking about the emerging infectious disease centers.

Mr. TOWNS. Yes.

Dr. SATCHER. Let's see if I can remember them. Connecticut is one. New York is one. California.

Mr. SHAYS. New York is one.

Dr. SATCHER. California has one. And I believe Oregon. Georgia now has a center. And I'm blocking—oh, Minnesota and Maryland.

Mr. TOWNS. Minnesota.

Dr. SATCHER. Those are the seven. And we do hope to fund one more in fiscal year 1998. And then we would like to move to maybe at least two more without addressing Emerging Infectious Disease Programs. So we have in the fiscal year 1998 budget plans for continuing to expand our Emerging Infectious Disease Programs throughout the country.

And as you know, the one in Connecticut was very helpful to us in looking at some of the issues related to the cases you'll probably be discussing later with some of the others. But it's been very helpful in terms of surveillance in that area.

Mr. TOWNS. Thank you.

One of you alluded to the whole HIV thing. Let me sort of—is there any similarity between the growth and spread of AIDS throughout the population and the growth of CJD?

Dr. SATCHER. I guess I would say very little. And let me just explain what I mean. If you look at the HIV epidemic, which we first identified in 1981 from epidemiologic data, we didn't identify the virus until 1983 in this country, the AIDS epidemic has spread rapidly throughout the world and is now a global epidemic or a pandemic.

It's an epidemic that continues to spread for many reasons. No. 1, the transmissibility of the virus. It's possible to spread this virus through the transfer of body fluids and that includes sexual intercourse and other ways in which body fluids are transferred, obviously transfusion; injection drug use. And so generally the transfer of body fluids makes spreading of HIV possible. We don't think that's true with CJD.

The other thing with HIV that's made it such an epidemic, of course, is the prolonged period of incubation before a person becomes ill in many cases, and the fact that all during that period of time, that person is capable of transmitting the virus to other people.

We get excited about Ebola when there's an Ebola outbreak because it's so dramatic. It kills 80 percent of the people it infects. But it cannot sustain an epidemic easily because it kills the host so rapidly that they don't have time to spread it to others. But HIV is just the opposite. People can walk around 5 to 10 years with the virus spreading it to others without being ill themselves.

Now, with CJD, we have conducted mortality surveillance since 1979 in this country. And we have seen no evidence of any major change in the fact that about 1 in 1 million persons is infected with CJD. There's been no significant increase. You know, that's been very important with the BSE out—problem in England, bovine spongiform encephalopathy. The fact that we've seen no increase and no change in terms of the age of persons with CJD has given us some comfort that we're not facing that problem. So CJD has been very stable and the preponderance of scientific evidence would suggest to us that it is not transmitted through blood.

However, as Dr. Brown said, things like being able to detect the prion in the blood of a very small number of persons with CJD concerns us and the fact that in animals you can, in fact, transmit the infection from blood if injected into the brain. But there is a big difference from HIV.

Dr. BROWN. And to Dr. Satcher, in the context of comparison between HIV and CJD, there are two things that we don't have in CJD that would be awfully nice to know. The first thing is that people with CJD are rarely found to be donating blood. People who are sick don't generally donate blood. We don't know how long before a patient gets CJD clinically he might be infectious. And we have no test to detect the infectivity in blood. Major difference with HIV. We can't screen for silent incubating CJD patients. And that would be a very useful thing to know.

Dr. SATCHER. Very good point.

Mr. TOWNS. All right. Let me just ask one more, and then I will move on.

Mr. SHAYS. Sure.

Mr. TOWNS. Dr. Satcher, I have read the public health system is in disarray. I understand that in some States and local health clinics the simple process of getting children vaccinated can become a long, long ordeal.

Do you have any suggestions for the Congress in what we need to do to assist in rebuilding our health system?

Dr. SATCHER. That's a very important question. And I hope I can do it some justice.

The Institute of Medicine's report in 1988 pointed out that our public health system was in disarray. We have allowed our public health infrastructure in many cases to deteriorate. I think we've seen that most dramatically in the resurgence of tuberculosis, a disease that we thought we had under control. But for many reasons, not just the deterioration of our public health infrastructure, but new changes like HIV and increasing immunodeficiency, for various reasons we saw the return of tuberculosis.

CDC has been committed now for several years to help rebuild the public health infrastructure by supporting State health departments, for example. And one of the things we're doing with the emerging infectious disease program, in addition to the emerging infectious disease centers that we have in a few States, we have now 20 States where we have been rebuilding the public health laboratories so that they can play a stronger role. We have the Public Health Leadership Institute, and we just initiated one in the Northeast with New York, Pennsylvania, Maine, Vermont, New Jersey and Rhode Island where we're training leaders in public health over a year's period of time. So that now in almost half of the States in the country there are public health leadership institutes.

We keep asking Congress for support that would allow us to rebuild a public health infrastructure so every year our budget reflects that goal.

Mr. TOWNS. Right.

Dr. SATCHER. And not just ours, we're working with our partners in FDA and NIH and others.

Mr. TOWNS. This question comes up, Mr. Chairman, in the question you raised you wanted additional information on. It is my understanding that although the FDA Blood Advisory Committee has considered pool size, it has not issued final recommendations about pool size.

Can you tell us where the FDA is in that process? If you want to add that to what the chairman is saying, we can do it at that time. I would like that to be a part of the question. And at this time I would yield back.

Mr. SHAYS. Dr.—Mr. Snowbarger.

Mr. SNOWBARGER. Not Dr. And I am going to prove that with the questions I asked. And I do apologize in advance for perhaps the ignorance of the questions, but I need to get back to a little more basic information. And prior to preparation for today's hearing I was not aware of CJD and don't know much about it. So I would like to ask some questions there particularly as it is related to the blood supply.

Am I hearing you correctly, and I am talking about you as a panel, that we are not certain the extent to which CJD is transmitted by blood or blood products?

Dr. SATCHER. I think, and Dr. Schonberger testified here in January and he is certainly one of the world's experts in this area, I think our conclusion would be that the preponderance of scientific evidence to date is that there is no evidence that CJD is transmitted in the blood. Having said that, CDC continues to look at this issue through retrospective studies. And to date, for example, there has been no evidence that persons with CJD are more likely to have hemophilia or sickle-cell disease or thalassemia where people receive a lot of transfusions. In fact, I don't believe there's been a report of CJD in a hemophilia patient in the world. And now of course we've initiated our prospective studies looking very critically in hemophilia patients and studying over a period of time to make sure that if there is any evidence out there that we will find it.

So on the one hand I'm saying there's no preponderance of evidence to date, and on the other hand I'm saying we're still looking openly and critically at this issue.

Dr. BROWN. Do you want a 33-second primer on CJD?

Mr. SNOWBARGER. Thirty-three would be about right. That's about all I can hold.

Dr. BROWN. CJD kills about 1 in 1 million people each year, which translates to about 250 to 300 cases each year in the United States. It starts out with forgetfulness, to which is added fairly quickly abnormal movements, visual deterioration, rigidity, mutism, blindness, coma, death. You can think of it as Alzheimer's disease in fast forward played out in about 7 months instead of 5 years.

Mr. SNOWBARGER. That is OK.

Mr. SHAYS. It is amazing. He did that in 33 seconds.

Mr. SNOWBARGER. You timed it.

What age patient does it normally affect, presuming it is not transmitted by blood?

Dr. BROWN. I understand. The average age is 60, right on the money for your present witness, and span about 15 years in either direction and you've got about 90 percent of all cases of the disease. So it's a disease of, well, I used to say old age, now I say middle age.

Mr. SNOWBARGER. I understand.

Dr. SATCHER. Let me just say, that's so important because there is the problem in England with BSE. The difference was, of course,

that younger people were getting this disease. And so we started to look to see if there was any change in this country in the age at which people were getting CJD and there was none. So that was very important.

Mr. SNOWBARGER. OK. I have heard the figures "one in a million" mentioned several times. And as it relates to the blood supply—well, first of all, my understanding is the one in a million refers to the number of people that have the disease or are diagnosed with the disease.

Dr. BROWN. The number of new patients that will appear each year so——

Mr. SNOWBARGER. OK. So we are sort of—as we talk about the blood supply, we are sort of transposing that figure over there and saying, well, if it's one in a million patients, then it may or—well, it is probably one in a million units of blood or one in a million donors.

Dr. SATCHER. No, I don't think so. Because I think because of the age of CJD persons and the fact that they're more likely to be ill, I don't think they're as likely to donate blood as an HIV patient, for example.

Mr. SNOWBARGER. OK.

Dr. SATCHER. So we don't think it would be one in a million.

Dr. BROWN. For your thinking in rough figures, you can stay with one in a million.

Mr. SNOWBARGER. But I think this is important. I mean, we are spending a lot of time, effort, research dollars paying a lot of attention to pool size, et cetera, on this particular—I mean, pool size affects other diseases, I presume. And I am aware of that. But talking about CJD, you know, is it 1 in 2 million? Is it 1 in 3 million?

Dr. BROWN. For donors?

Mr. SNOWBARGER. Yes. I mean if we are saying that you have got one in a million of the general population—I guess what I am looking for is if we have got any kind of guess about what it would be in the donor population.

Dr. BROWN. Well, I think the answer you're looking for is known. And that is that in studies, large epidemiologic studies in Europe and a couple of other places in the world, as it happens, looking back, if you ask what proportion of patients dying of CJD have at any given time donated blood, it's exactly the same proportion as the general population.

Mr. SNOWBARGER. OK. And because we don't know whether it—it lays there dormant as a factor in the blood, we have to assume it is one in a million; is that what you are trying——

Dr. BROWN. That's right.

Mr. SNOWBARGER. I am kind of like a chairman, I have to have it explained in my terms or I don't understand it. So I understand what you are saying.

Now, it is my understanding that it is very, very difficult to diagnose CJD.

Dr. BROWN. It used to be. I think it is no longer. And the diagnosis of CJD has now, as we speak, achieved an extraordinarily high degree of accuracy.

Mr. SNOWBARGER. And how is it diagnosed?

Dr. BROWN. You usually diagnose it clinically. There are not too many diseases with which it can be confused. I mentioned Alzheimer's disease. That's always an issue for a little while. But Alzheimer's disease tends to stretch out for 5, 6 years; whereas CJD usually kills within a year and typically within 4 or 5 months, a much faster evolving disease.

Mr. SNOWBARGER. So we are saying that the main method of diagnosis is how fast you die, not if you die?

Dr. BROWN. No.

Mr. SNOWBARGER. And you are finally diagnosed when you actually hit the end?

Dr. BROWN. Well, pathologists will say "right on" because they always like to make the final diagnosis. But in point of fact you can diagnose it clinically by the kind of evolution and by the combination of symptoms. The pathology of the brain will put the stamp on it. And there are biologic tests which will, also. But it's not a difficult disease to diagnose either clinically or at autopsy.

Mr. SNOWBARGER. And you are still in the process of researching whatever factors that would allow us to begin finding the same factors in the blood supply or do we have any way to do that?

Dr. BROWN. Well, there's enough unknowns about this field and the disease that can sustain further careers, and the blood supply or the issue of CJD as a risk in causing CJD through the blood supply is one of the issues that we are discussing this morning.

Mr. SNOWBARGER. OK.

My last question, Mr. Chairman, because I am interested in the answer to your question, because I think that is the crucial question, of pool size and how we deal with that. Let me ask two questions, if I may.

One is, it sounds to me like in terms of CJD, not looking at other infectious diseases, but in terms of CJD, the ideal pool sizes are either one or infinity, not somewhere in between. I mean that would be the best of all worlds. Because if you have it in one—I mean, if we do it in pools of one unit, which we are going to do, but I mean, if you do it in pools of one unit, then you can isolate, you know, one to one. And if you have an infinite supply, then what you are saying is that that may dilute these factors.

Dr. BROWN. Yes. That's a very intelligent summary, Mr. Snowbarger. You start with——

Mr. SNOWBARGER. Well, I appreciate that. It doesn't lead us to any conclusions unfortunately. Nice academic question. But I mean——

Dr. BROWN. Yes, if you give a contaminated donation to one recipient, you can never cause more than one infection for sure. It is possible that your pool of infinity might dilute out infectivity to zero. But only if it takes two or three particles to make a single infection. Then as you diluted it out to infinity you would have less and less chance of having those three particles together. But that's not a fact that is known. We don't know how many particles it takes. And if it only takes one, then when you dilute out, you will get down to the threshold plateau minimum and that will stay the same until infinite. So I don't think we can properly say that that's not an important conclusion.

Mr. SNOWBARGER. Well, we are going through a lot of math exercises today which may or may not help, I don't know, but I want to ask a question that I understand may seem very callous. We are going through a period of time where we are trying to do cost-benefit analyses on things that we do and things that we get involved in. And the figures that I have heard today is that, No. 1, we don't have any clinical evidence that CJD is passed on by blood products. And No. 2, the risk is at about one in a million, at best from what we know.

Could you give me your observations about the cost-benefit analysis of all the effort that we are going through? And, again, I understand that is callous, particularly for those who either suffer the disease or have loved ones that do, but in trying to get a handle on priorities, there are certainly other diseases out there that have a much higher risk for the population as a whole than one in a million.

Anybody care to comment on that or are you just going to leave me hanging out there with my——

Dr. BROWN. I won't leave you hanging, Mr. Snowbarger. Obviously, there is what is trendily called "cost-benefit" to everything we do. And when we wake up in the morning, we're taking a risk. We can minimize the risk as best we can, and that's going to cost money. But I really have no feeling about where the line should be drawn in this particular disease. As a career research scientist working on the disease, I would say keep spending.

Mr. SNOWBARGER. Sure. I understand. I didn't expect an unbiased answer, but the reason for my question is that, as you said, we all take risks every day. And there are certain risks that—and a certain level of risks that we have all come to be willing to accept, the risk of an automobile accident, the risk of slipping and falling in a bathtub. You know, most folks don't stay out of the bathtub because they might slip and fall, and most of us still ride in cars and stuff like that. And, again, it is a callous approach to it. Any cost-benefit analysis is a callous approach. I am not suggesting necessarily we take that approach here, but I do think that at some point we have to figure out how to allocate scarcer and scarcer resources for the things.

Mr. SHAYS. Let me say to you it is not a callous question. I have been in public life for 24 years. And in the State House. I would continually have people say, well, if it saves one life, you should do it. And then you don't even—that's the argument. But we could save a lot of lives by making the speed limit 25 miles an hour. So we make certain decisions whether we care to acknowledge it or not.

I am going to want to get an answer, not a long answer, to the advantages and disadvantages, just so I have it outlined. But I would like to call on Mr. Towns, and then we will come back to that.

Mr. TOWNS. Two things. One came out of the dialog between you. But this is a diagnosis that is easy. So help us here because we need to know as much as we can, as much of a basis as we can get.

As people begin to live longer, and thank God that that is happening, will the diagnosis then be harder to make?

Dr. BROWN. Yes, Mr. Towns. It will be a little harder, because a major feature of CJD is mental deterioration, senility, if you like.

Mr. TOWNS. Right.

Dr. BROWN. You know, and Aunt Emmy starts to forget at the age of 83, she might be coming down with CJD, or she might just be getting old. The answer is usually what physicians describe as the fourth dimension, which is time. If she's getting old, she'll probably get old fairly slowly. If she's got CJD, she's likely to be dead in 6 months.

Mr. TOWNS. We will hear testimony about a case of a young hemophiliac who died of HIV-related causes. Because of receiving a letter from the FDA, his grandmother suspected CJD involvement at his death. After the autopsy and additional information by CDC, CJD was ruled out.

This question is for the entire panel. It seems that some would point to this as a failure of the system, but it seems to me that there was notification and investigation by the Government agencies, so that is a question. I think the failure here was that the child died of HIV-related causes.

Can someone here tell me what has been done about the HIV exposure, what steps were taken once it was realized that this child died of HIV, which presumably was contracted through the blood products he used? Either one of you can start.

Dr. SATCHER. Let me just briefly comment on the HIV exposure. I think, as you know, early in the epidemic there was really an unfortunate situation with the number of people who were exposed to HIV through blood transfusions. Since that time we've made a lot of progress in terms of donor screening and viral inactivation. So it would be very unusual for a person with hemophilia to acquire HIV through routine transfusions because of what we have learned. But that was a very unfortunate part of our history in public health any way you look at it.

And so our sensitivity and our concern in this area is very high, very deep. We don't claim to understand all of the aspects of this one case in terms of the system of surveillance. As you pointed out, we have had pathologists to examine slides of tissue and the conclusion was that this was not CJD, but still the child had HIV and there were some neurological concerns. So it was not reported to CDC by the physician as a case of CJD or suspected CJD.

Mr. TOWNS. Yes.

Ms. ZOON. Yes. From the FDA's perspective, the safeguards and the tests, donor screening testing methods, viral inactivation have been a focus of activities since the AIDS epidemic. And every step is being taken with the team of the Public Health Service, the cooperation between the research being done at the National Institutes of Health and others, and the surveillance efforts of the FDA to make the maximum use of the information that we get to develop and additional safeguards, if possible, for blood safety as it comes to HIV.

Like Dr. Satcher, without the particulars of the case, I cannot comment more specifically about this unfortunate incident, but we are committed to working very hard to improve systems that have been dramatically improved, even more as the technology evolves.

Mr. TOWNS. Mr. Chairman, I yield back.

Mr. SHAYS. I don't want to spend a lot of time on this because I want to get to our next panel, but I want a list of the advantages and the disadvantages of a large pool size.

We will first take the advantages. Dr. Zoon, why don't you start?

Ms. ZOON. I will start and give Dr. Satcher a break. He has been starting all of the questions.

I would like to start—what I will do is I will list advantages and disadvantages that I see for larger pool sizes.

The disadvantages that I see are the spread of infectious risks over more recipients for a single lot with a larger pool size. Second, large single lots will cause bigger recalls or withdrawals, depending on the size.

Mr. SHAYS. These are disadvantages?

Ms. ZOON. Disadvantages. I think there is an increased exposure risk to infrequent recipients with larger pool sizes.

Mr. SHAYS. Explain that one?

Ms. ZOON. An increased exposure risk to infrequent recipients. That means if you are getting just a product once, versus someone who is taking product daily or frequently for management of a disease. If I were in a car accident and it was a one-time recipient, that would be an infrequent exposure.

Mr. SHAYS. Right. I understand that part. I don't understand how it relates to the disadvantage. What is the disadvantage here? You have given me one, the spread; you have given me the recall.

Ms. ZOON. I said, with larger pool sizes, there is an increased exposure risk. So it is a disadvantage because you have a greater probability of being exposed.

Mr. SHAYS. I understand that. OK. What is the next one?

Ms. ZOON. OK, the next one would be it could accelerate in the case of a new emerging infectious agent the spread of an epidemic.

Mr. SHAYS. That I would think would be one of the most alarming ones, obviously. In other words, basically an infection we have never even considered in a large pool size.

Any other disadvantages?

Ms. ZOON. Those are the major ones.

Mr. SHAYS. What would be the advantages?

First off, would we add any other disadvantages, Dr. Brown or Dr. Satcher? You have given us four.

Dr. BROWN. You are going to get this, I am sure, from the industry tomorrow. Obviously——

Mr. SHAYS. Tonight. Sorry, this afternoon—not tonight.

Dr. BROWN [continuing]. It is the cost-benefit argument again. I am sure you will hear economic arguments, and I would suppose if we were making a list——

Mr. SHAYS. We are talking disadvantages.

Dr. BROWN. OK.

Mr. SHAYS. The larger the pool, the disadvantages. I think we accept those disadvantages.

Let me talk about the advantages. What are the advantages? Dr. Zoon, since you started?

Ms. ZOON. Sure. One advantage, potentially, could be manufacturing efficiencies. Another could be possible neutralization of an agent. Often there are antibodies present, and having more donors in a larger pool size could have some neutralizing agents in them.

Mr. SHAYS. Could the opposite happen?
Ms. ZOON. Could the——
Mr. SHAYS. Opposite of neutralization happen.
Ms. ZOON. You mean enhancement?
Mr. SHAYS. Yes, enhancement. You didn't mention that in your four. I am wondering if we could add that to No. 4.
Ms. ZOON. It is theoretically possible.
Mr. SHAYS. OK.
Ms. ZOON. Possible dilution below the——
Mr. SHAYS. Let me say, if we are going to use the same judgment, is it theoretically possible of neutralization? Or is that more established that there is a concept?
Ms. ZOON. There is more established data.
Mr. SHAYS. Neutralization is more established than enhancement.
Ms. ZOON. Correct.
Mr. SHAYS. OK.
Ms. ZOON. Possible dilution to extinction. This refers to the situation that Dr. Brown——
Mr. SHAYS. I am with you.
Ms. ZOON [continuing]. Referred to. Enhancing genetic diversity in the product. This would be particularly important, perhaps in the area of immunoglobulins.
Mr. SHAYS. OK. That is helpful.
I am going to ask this question just because we need it for our record and our report. This would be addressed to both of you, Dr. Satcher and Dr. Zoon. Is the FDA position on withdrawal of products manufactured with plasma of donors infected with, or at risk of infection with, CJD still prudent public health policy in light of current research on CJD?
Dr. Zoon.
Ms. ZOON. As Dr. Satcher mentioned, at this time there is no evidence of a body of data suggesting that transfusion of blood products to humans results in a case of CJD.
As Dr. Brown has discussed, there are experiments under way helping us to further evaluate in a variety of animal models the risks associated with fractionated products using these animal models to have a sense of what that is.
At this time, the agency believes that we should be looking at this very carefully and very closely and be ready to act more aggressively as data is generated. I think this is a case where the Public Health Service has discussed this very frequently. We have brought it to several of our advisory teams.
Mr. SHAYS. You remind me of the State Department here. I need to get the answer.
Ms. ZOON. Oh. I think at this time we believe it is still considered a withdrawal. However, we are constantly poised to re-evaluate that situation.
Mr. SHAYS. Fair enough. Dr. Satcher.
Dr. SATCHER. I agree. The only thing I would add is I think the action of this committee, I believe, established the blood safety committee of our Department, and now we have established the Advisory Committee to the Department. I think some of these issues, as time goes on, will be discussed with the Advisory Com-

mittee. The perspective has to be broader than those of us who work in public health that on a day-to-day basis. There are some values involved. Some of these things will be taken to the advisory committee.

Mr. SHAYS. Before we go to the next panel, a very active member of the committee, Dennis Kucinich, is here and has a question to ask. Then we will go to the next panel.

Mr. KUCINICH. This is to Dr. Brown. Thank you very much, Mr. Chairman.

In reviewing your testimony, Dr. Brown, I am impressed by your comments relating to the situation with respect to a donor pool which contains a large number of infectious particles and also to your, if I may say, tentative conclusion that the chance of contracting CJD from a pooled blood product in which a patient with CJD has contributed is extremely small, no matter the size of the donor pool.

That, of course, assumes, for the sake of the study, that the only pathway you are looking at is the pooled blood supply with respect to this study.

In looking at this overall issue, Mr. Chairman, it occurs to me that what we are talking here about CJD is synonymous, is it not, with the bovine spongiform encephalopathy, popularly known as "mad cow disease," which has resulted in a pathway of transition being consumption of food products?

Would it not then be true that the consumption of food products in a given population that would be contaminated with the BSC, bovine spongiform encephalopathy, CJD, whatever you want to call it, does that increase the possibility of contamination of the blood supply? And then is it not true that protocols for prevention of such contamination to the blood pool would necessarily include contact with those agents which transmit the disease through the food supply?

And the final part of the question, a small question——

Mr. SHAYS. He said he had one question. It is going to take an hour to answer this. That is cheating.

Mr. KUCINICH. In line with your question, have you had contact with the State Department and the U.S. Department of Agriculture about these things?

Mr. SHAYS. I am going to give you each a minute.

Mr. KUCINICH. What are you doing for lunch?

Mr. SHAYS. I am sorry to interrupt you.

Mr. KUCINICH. Thank you. That is fine.

Dr. BROWN. Your analysis is absolutely correct.

Mr. SHAYS. He was just showing off. We can really go on to the next question.

Dr. BROWN. It is one of the things that the United Kingdom is currently concerned about.

Let us suppose that instead of 20 cases of the new variant, which they now have, and let us further suppose that the new variant is the result of consumption of tissues from animals with the "mad cow disease," instead of 20 cases, suppose in the next year they have 2,000 or 20,000 cases? Nobody knows whether that is going to happen yet. If that happens, you have augmented the potential contamination of your population that is donating anything, wheth-

er it be blood, dura mater, kidneys or any other tissue, by 100 times.

So, again, that is a correct analysis; and it is something that is very worrisome to the United Kingdom and also which we are studying at the NIH. Because, again, we would like to prevent things and not just say, "oops, we didn't see it in time."

Mr. SHAYS. I am going to just make sure I have this on the record. Is the gentleman done?

Mr. KUCINICH. Yes.

Mr. SHAYS. Do you all agree that a lot size of 400,000 is far larger than it needs to be? I don't want to put words in anyone's mouths here, so I don't want you to say "yes" if you don't think that. We were surprised with a lot size of 400,000.

Let me put it this way, since I didn't get a quick answer to that. Do we agree that there are benefits in having some standard sizes? Dr. Satcher.

Dr. SATCHER. Yes, we think there are benefits to looking at this issue and trying to arrive at some limits in terms of size.

Mr. SHAYS. Not just limits but also that there would be—we could learn things from having standard sizes when we encounter certain problems and then can maybe compare, as opposed to having them all over the lot.

So I am asking specifically, No. 1, should there be an upward limit; and, No. 2, should there be some standard sizes? Is there an advantage in having some standard sizes?

If you don't know——

Dr. BROWN. No, really, we have standards for everything else in the world. I suppose we could have standards for lot sizes. I am not trying to be facetious, but I can't at the moment think of any advantage to having a standard lot size.

Mr. SHAYS. OK. Let me ask you—fine, I don't mean to stretch this out, but—yes, Dr. Zoon?

Ms. ZOON. Yes. I think that the considerations will need to be on a product-by-product basis, and that is the analysis we are hoping to do. Clearly, for products like immunoglobulins, you would like to have diversity in the pool; and, actually, there is a regulation of a minimum donors per pool for that particular type of product.

So I think the answer to your question is twofold: One is, should there be an upper limit to the primary pool; and then looking at the fractionation of each of the separate products that would need to be analyzed and looked at very carefully, and we are in the process of collecting the data and evaluating that data.

Mr. SHAYS. OK. I am just going to say, for the record, this committee is not about to try to push the FDA, the CDC or any part of the National Institute of Health in a direction that doesn't make sense. As we look at it, though, we do see there has got to be some ultimate limit, unless it can be proved that there is a reason to do it. I mean, that is where this committee is headed. We will be interested in testimony from others as it relates to that.

You have been a wonderful panel. I am going to do this at risk. There were about eight people who stood up behind you who were sworn in. But if any of you just felt there is something we really need to put on the record and you are willing to show courage and risk offending your bosses for the good of humanity, I would love

you to just feel free to come forward. I sometimes have been in your position and say, why the heck didn't they say that?

Is there anyone who just wants to make a point? I am being serious. We would welcome it.

OK. Thank you. Thank you very much.

[The information referred to follows:]

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July 30, 1997

The Honorable Christopher Shays
Chairman
Subcommittee on Human Resources
Committee on Government Reform and Oversight
Room B-372 Rayburn House Office Building
Washington, D.C. 20515

Dear Mr. Chairman:

I am responding to your July 16 letter to Dr. Varmus inviting testimony from Dr. Paul Brown of this Institute on the subject of the risk of infectious disease, particularly Creutzfeldt-Jacob disease (CJD), associated with pooled plasma products. As the official responsible for resource allocation within the National Institute of Neurological Disorders and Stroke (NINDS), I am pleased to respond to your request for information about future plans for research on CJD and other transmissible spongiform encephalopathies (TSEs).

The Laboratory of Central Nervous System Studies (LCNSS) has a long-standing interest in this area of research, dating from studies in the1950s and 1960s that established the existence of a novel class of slow-acting infections of the central nervous system, now known as the TSEs. The laboratory is also involved in the study of nontransmissible dementing disorders such as Alzheimer's disease as well as a broad array of unusual infectious brain diseases seen all over the world.

As you may know, each intramural research project at the NIH is reviewed every four years by the Institute's Board of Scientific Counselors (BSC). Based on their evaluation of scientific excellence and programmatic considerations, the BSC may recommend changes in emphasis or resource allocation. At the most recent BSC review of the Laboratory of Central Nervous System Studies in January 1995, Dr. Carleton Gadjusek, the Chief of the Laboratory at that time, indicated his intent to phase out the Laboratory over a five year period. This decision met with the full concurrence of the Board of Scientific Counselors who acknowledged the Laboratory's past contributions and made explicit recommendations about how the phase out could be best accomplished. In particular, the BSC had recommended that Dr. Brown complete projects ongoing at the time of the review and that he be encouraged and supported in his role as an expert advisor to national and international bodies.

Dr. Gadjusek has since retired and the Laboratory, under the direction of Dr. J. Clarence Gibbs, Jr., Acting Chief, has focused its efforts almost entirely on TSEs. In keeping with recent developments concerning TSEs, the original plans for reducing support for the Laboratory have been revised and support is presently stabilized at the fiscal year 1997 level. The Laboratory will have an estimated operating budget for the coming fiscal year of $2.2 million, including support of primate studies, and 15 investigators and support staff. We anticipate that support will continue at this level for two years and further support could be extended if the research projects underway warrant an extension and review by the Board of Scientific Counselors supports such a commitment. Dr. Brown recently began a final three year appointment in the Public Health Service Commissioned Corps, bringing his tenure in the Corps to the maximum allowable 36 years as of 8/1/99. After the completion of this tour of duty in the Corps, there are other appointment mechanisms available should it be mutually advantageous for Dr. Brown to continue his research here.

In closing, I would like to point out that the interest of this Institute in transmissible spongiform encephalopathies such as CJD extends beyond the research being conducted by the LCNSS. Our Institute supports a substantial extramural grant program in TSEs. Total NINDS funding for FY 1997 is estimated at over $7 million. Other Institutes also support work in this field. The LCNSS works closely with the National Heart, Lung, and Blood Institute (NHLBI), which has funded some of Dr. Brown's work. The laboratory also collaborates with the Centers for Disease Control and Prevention (CDCP), the Food and Drug Administration (FDA) and the American Red Cross.

I hope this statement is helpful. Please do not hesitate to contact me if you require additional information.

Sincerely yours,

Zach W. Hall

Zach W. Hall, Ph.D.
Director

cc:
Dr. Varmus

Mr. SHAYS. We are going to call our next panel. This is Dolores Crooker, a parent, a registered nurse and a grandparent; Dr. Glenn Pierce, National Hemophilia Foundation; and Charlotte Cunningham-Rundles, Immune Deficiency Foundation. If they would come forward.

I am going to say for the record that Mr. Snowbarger just wants to kind of contradict something I said. I really would like it part of the record just because I think it is important to see diversity on this committee.

Mr. SNOWBARGER. Well, I noticed that the chairman asked those who were sitting behind their bosses if they cared to contradict or say anything that their bosses might not appreciate, but he didn't ask me if I wanted to contradict what the chairman had said.

At the risk of offending my chairman—and I know he will take it in the right way—I was a little concerned about the comment that the chairman said about the committee's direction. It left me with the impression that the committee is attempting to say that there needs to be—we need to find the optimum pool size and, once we find it, we need to make sure everyone is adhering to that. When what I heard from the witnesses sort of led me to the indication that there may not be an optimum pool size.

If there is not an optimum pool size, then why do we care? And maybe in this era of deregulation it might be a time to think about maybe it is something we don't need to get into, as opposed to trying to find every little place where we can do what we think is best, where the industry, particularly if the regulators don't see any particular need for it, we don't need to push them into regulation.

That was my comment.

Mr. SHAYS. I appreciate your putting that on the record.

What I really should say is that that was the position of the committee last year; and, obviously, there is no reason why we shouldn't revisit it. It was in a report recommending there be a limit. But this is why we have hearings. So I am happy you are putting that on the record.

Mr. SNOWBARGER. Thank you, Mr. Chairman.

Mr. SHAYS. I am going to ask the witnesses to stand up and raise your right hands.

[Witnesses sworn.]

Mr. SHAYS. All three have responded in the affirmative.

STATEMENTS OF DOLORES CROOKER, R.N., PARENT; GLENN PIERCE, M.D., Ph.D., NATIONAL HEMOPHILIA FOUNDATION; AND CHARLOTTE CUNNINGHAM-RUNDLES, M.D., Ph.D., IMMUNE DEFICIENCY FOUNDATION

Mr. SHAYS. We will just go right down the row, starting with you, Ms. Crooker.

Ms. CROOKER. Good morning, Mr. Chairman.

Mr. SHAYS. I am sorry. I should tell you to move the microphone. I know it is hard for you to put papers in front of you and do that, but that is not going to be close enough, Ms. Crooker. Then you can turn it sideways a little bit. Turn that a little sideways a little bit. I am going to have you lower it down a little bit.

Ms. CROOKER. OK. Good morning, Mr. Chairman and committee members.

My name is Dolores Crooker. I am a grandmother and was the caregiver of Roger. I had taken care of him since he was 18 months old and diagnosed with severe hemophilia in 1984.

Roger died on June 21, 1995, of AIDS complications at the age of 11, 1 month short of his 12th birthday. In fact, yesterday would have been his 14th birthday. He became HIV infected from contaminated blood products he used to control his bleeding problems.

In 1993, Roger received a Factor VIII infusion in a local emergency room for an injury he received. This was recalled in 1994 for possible contamination with CJD. One year from product dispensing to community notification.

In late summer 1994, Roger came down with a severe neurological motor coordination problem. He improved for a short time and then continued to deteriorate until his death in 1995. No one could definitely identify the specific reason for his neurological deterioration, which included muscle spasms, seizures, blindness. It was 5 months before his death when I received notification that he had received this recall factor. This is 2 years after he received the factor.

Roger died at home on June 21, and an autopsy was performed. The first autopsy results showed spongiosis cells, and a second opinion was requested. In that report, it was suggested that a special protein stain be used. After a letter from me, the slides were then sent to the CDC for evaluation.

I know that Roger died of HIV infection he contracted from the blood product he used since his diagnosis of HIV in 1984, but was CJD present? Were these spongy cells caused by HIV or CJD? I had to know. It was about 2 years before I knew he had received the recall factor and now 2 years after his death I finally got the answer that CJD was not present.

I am not aware of what reporting methods were used by the various medical/scientific communities to study this unusual autopsy report. I do feel, however, that a final answer to myself for closure and peace of mind—that took too long.

As a member of the hemophilia community, I should not have to tell you how important it is to explore and analyze matters such as this in a very timely fashion. In the 1980's, we waited long periods of time and lost precious years waiting for the final answer on the question of HIV and AIDS and blood products transference. As we are aware, the answer then was positive; and the devastating epidemic took more than 50 percent of our community.

We cannot afford to waste more years at a tragic cost and more lives on finding out answers regarding CJD and other transference.

It took over 2 years since my grandson's death to get a definite answer on his disturbing autopsy. My anxiety and my concern are not for my peace of mind only. This community, including the new generation so far untouched by HIV and hepatitis, needs to be completely informed about the hidden perils lurking in an FDA-approved product they continually use. Product recall notification must be faster than 2 years. It was 2 years to find out he received recall product and 2 years to find out the autopsy results.

Faster recall notifications have occurred within the past few years because of collaboration with community organizations, the treatment centers and Government regulations—and some Government regulations.

In closing, I feel that it is imperative that all departments of the medical community—clinics, hospitals and labs—should have a common goal: find answers quickly and relay this information accurately to the waiting family members. Unfortunately, delay may cost additional lives.

In my case, 2 years is 2 years too long. If the Government is going to approve a Federal product through the FDA, then it should also take the responsibility to regulate procedures for recall and withdrawal of that product, because contamination and even potential hazards can cost human lives.

Thank you.

Mr. SHAYS. Ms. Crooker, it is so important that you provide a human face to what we are talking about. We can just talk about statistics and numbers and so on, and it is just very welcome that you would express your concern. You cared for your wonderful grandson for 11 years of his life, and so we just really appreciate your being here.

Ms. CROOKER. Thank you.

Mr. SHAYS. Are you accompanied by Roger's sister?

Ms. CROOKER. His sister is over here.

Mr. SHAYS. It is nice you are as well.

Ms. CROOKER. His younger sister.

[The prepared statement of Ms. Crooker follows:]

Good Morning Mr. Chairman & Committee Members, My name is Dolores Crooker. I am the grandmother and was caregiver for Roger. I had been taking care of him since he was 18 months old and diagnosed with severe hemophilia less than 2% clotting factor in late 1984.

Roger died June 21, 1995 of AIDS complications at the age of 11, one month short of his 12th birthday. Yesterday should have been his 14th birthday. He became HIV infected from contaminated blood products used to control his bleeding problems.

In 1993 Roger received a factor VIII infusion at Robert Wood Johnson University Hospital for an eye injury. November 18th 1994 a Medical Bulletin from the Hemophilia Association of NJ informed the community about a recall & withdrawal of certain lots of American Red Cross plasma derivatives which include the anti hemophilia-factor (AHF). This withdrawal was because of possible contamination of Creutzfeldt-Jakob disease. It was also known as MAD COW disease. *Over one year from product dispensing to community notification.*

In the late summer of 1994 Roger came down with severe neurological/motor coordination problems. He improved for a short time then continued to deteriorate until his death in June 1995. No one could definitely identify the specific reason for his neurological deterioration which included muscle spasms, seizures & blindness.

On January 31, 1995, 5 months before Roger's death, I received a notice from Dr. Saidi, Director of New Jersey Regional Hemophilia Program at Robert Wood Johnson, this notice stated that he received the factor VIII lots involved in that recall. *this is two years after he received the factor*

After several conversations with Dr. Saidi, Dr. Murphy, (Roger's pediatric hematologist) and the nurse coordinator at the treatment center, I did consent to a limited autopsy. Because of Roger's deteriorating condition this was quickly put into place.

Roger died at home on June 21, 1995. During October, 1995 I had more conversations with Dr. Saidi about the preliminary results of Roger's autopsy by attending Pathologist Dr. Schwarz at Robert Wood Johnson. For brief explanation, Robert Wood Johnson is a University Medical Center of which the Treatment Center is a distinct and separate entity. The Hemophilia Treatment Center referred the autopsy to the Pathology dept. They are not a part of CDC funding. The results showed spongiosis cells which Dr. Schwarz stated were due to the HIV infection, he ruled out CJD.

A second opinion was solicited by Dr. Saidi from Dr. Sharer, the Associate Director, Division of Neuropathy at the University of Medicine and Dentistry of New Jersey (UMDNJ). He has looked at many slides from the CDC dealing with CJD. He suggested a special protein stain (immunohisto-chemical stain for prison protein) he used. I received his reply dated February 15, 1997 in May of 97-I phoned Dr. Schwarz, the original Pathologist, about this report. Dr. Sharer had highly recommended the

slides be sent elsewhere for further review and analysis. After an unsatisfactory conversation, I was unsure when and if the slides would be prepared for this study. They were however, at Dr. Saidi's urging and a follow up letter from myself, sent to the CDC for evaluation.

I know that Roger died of HIV infection he contracted from the contaminated blood product he used since his diagnosis of hemophilia in late 1984, but was CJD also present? Were these spongiosis cells caused by HIV or CJD? I had to know!

It was about 2 years before I knew he had received the recalled factor & now 2 years after his death I finally got the answer that CJD was not present.

I am not aware of what reporting methods were used by the various medical/scientific communities to study this unusual autopsy report. I do feel, however, that a final answer to myself (for closure and piece of mind) took much longer than I expected.

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We cannot afford to waste more years at the tragic cost of more lives on finding answers regarding CJD and blood transference.

It took over two years since my grandson's death to get a definite answer to the disturbing finding on his autopsy. My anxiety and concern are not for my peace of mind only. This community, including the new generation so far untouched by HIV or hepatitis, needs to be completely informed about the hidden perils lurking in an FDA approved product they constantly use. Product Recall notifications must be faster than 2 years. It was 2 years to find out he received the recalled product and two years to get the final result of his autopsy.

Faster recall notifications have occurred within the last few years because of collaborations by the community organizations and hemophilia treatment centers, not government regulations.

In closing, I feel it is imperative that all departments of a medical community (clinics, hospitals, lab technicians, etc.) should have a common goal--find answers quickly and relay this information accurately to the waiting family members. Unnecessary delays may cost additional lives.

In my case, two years is two years too long! If the government is going to approve a product through the FDA, then it should also take the responsibility to regulate procedures for recall and withdrawal if the product becomes contaminated or even potentially hazardous to people's lives.

Mr. SHAYS. Dr. Pierce.

Dr. PIERCE. Thank you for this opportunity to present testimony today on behalf of the National Hemophilia Foundation.

The NHF is a voluntary health organization dedicated to improving the health and welfare of people with hemophilia, von Willebrand's disease, and other coagulation disorders. I am a former president of the Foundation and currently serve as the chair of its Blood Safety Working Group. I am a person with hemophilia and, as a result, have been exposed and infected by many viruses and other agents through my use of blood clotting factor. In my professional life, I manage research projects for a biotechnology company.

The NHF appreciates the continued efforts of Chairman Shays and this subcommittee in bringing greater attention to the critical need for safer blood products and a safer blood supply.

The Government Reform and Oversight Committee's 1996 report included two critically important recommendations which I will discuss today: reductions in plasma pool size and prompt patient notification.

The NHF has issued 12 medical bulletins in 1996 regarding products investigations or recalls and already has issued 12 bulletins this year, including 4 alone in July. In addition to the withdrawal or recall of products related to evidence of infectious agents, we are alarmed by the number of recalls this year that have resulted from violations in the FDA's good manufacturing practices where sterility was not maintained, vials were mishandled or viral inactivation did not occur as specified. Even recombinant DNA produced/non-blood based products have been recalled. Although these products are highly unlikely to contain human viruses, potential mold contamination during manufacturing resulted in a recall just last week.

Historically, the hemophilia community has been impacted by a number of viruses through the blood supply. While HIV has been the most devastating, a number of other viruses continue to plague the hemophilia community through their sequela, including Hepatitis A, B, and C and Parovirus B19.

Strong evidence of the need for a more responsible and responsive blood safety system accumulates as new announcements of blood product recalls are issued, often weeks after the seriousness of the problem has been detected. Too frequently, individuals in our community find out too late that they just infused themselves or their child with a recalled product which has been stored in their home refrigerators.

It is important, as we consider plasma pool size, to be more precise in defining the term. That has been a part of the problem, I believe. Plasma pool refers to the plasma donations that are mixed together for subsequent manufacture into purified coagulation products and immunoglobulins, albumin and other products.

During the manufacturing process, multiple batches of plasma may be mixed together, as we have heard this morning. The purified product is packaged and distributed in what is referred to as a lot. For some, but not all products, each lot is given a unique number to facilitate tracking. Thus, multiple pools make a lot. It is the final lot size that is of concern to the bleeding disorder com-

munity as lot size represents the total donors to which blood recipients are exposed.

We were both surprised and troubled to recently learn that there are no upper limits on the number of donors contributing to plasma lots. While manufacturing practices differ, we had been led to believe by FDA and manufacturers that manufacturers were observing limits of around 15,000 donors per lot for paid plasma and 60,000 donors per lot for volunteer plasma.

We now know from testimony this morning that some manufacturers place well over 100,000 separate donations in a single lot of products. The absence of any upper limit on lot size places our community at significant risk for emerging infections.

There have been attempts over the past year that have been made to pit the Immune Deficiency Foundation against the NHF on this issue. I will tell you categorically that will not work. Immunoglobulin and coagulation products are separated early in the manufacturing process. Thus, the needs of both groups in terms of final lot size do not impact on one another. This has been a smoke screen, and there has been deliberate obfuscation by industry on this issue.

In its own analysis of the issue, the FDA has published an article last year that made the case that larger plasma pools do increase the risk of exposure to and thus the risk of transmission of infectious agents, especially to highly susceptible populations, such as persons with hemophilia. Past experience with hepatitis and HIV in our community has demonstrated that not everyone who received a contaminated lot of product becomes infected as is assumed in the transfusion article.

We need only look at the spread of HIV to realize that patients received multiple exposures of HIV before becoming infected. Exposure to fewer donors would have allowed some individuals to escape infection.

We have communicated to FDA our requests for limits on the maximum number of donors that can be pooled together in the manufacture of blood products for the following reasons.

First, increased safety. As future emerging infectious agents threaten the blood supply, reductions in pool size can delay the possibility of widespread transmission.

Second, reduction of exposures. By reducing the total number of donors that a person is exposed to over a period of time, the likelihood of transmission of an infectious disease is minimized. As we heard this morning, this point is especially important for individuals who take product infrequently, who don't take it once every 2 or 3 days, but who may use it once a month or once every 2 months.

Finally, preservation of the product supply. Under the current situation, identification of a single blood donor with a disease can result in the recall of thousands of vials of clotting factor concentrate. Smaller pool sizes and placing donations from a single individual into a single lot, multiple donations from a single individual into a single lot, would do a lot to alleviate the amount of product that is withdrawn.

With regard to CJD, we have recently had numerous recalls due to possible CJD contamination. As this committee knows from its

previous consideration of the issue, we don't know if CJD can be transmitted by blood products, although the experimental evidence in animals suggests there is something to be concerned about there.

The number of recalls that have occurred because a donor was later identified as having CJD or at risk for CJD is staggering and has affected the supply of coagulation products in the marketplace. Clearly, if pool size limits were in place, substantially less product would be recalled.

With regard to patient notification, we know that recalls occur when the system of donor deferral, donor screening, viral inactivation, coupled with good manufacturing practices, is broken down. Frequent occurrence of these events at 1 to 2 per month on average continues to shake the confidence of consumers and providers within our community.

As a result of the events of the 1980's, where nearly half of our members were infected with HIV, we are committed to ensuring that consumers have information about the products they are using in order to make informed and educated decisions about their treatment. We believe this is only possible when they are provided with crucial and possibly life-saving information as soon as possible after an FDA investigation of an adverse event begins.

Since the announcement last year that notification should go down to the level of consumers by the FDA, the FDA has requested that companies with products in question contact consumer organizations such as the NHF; and we have issued medical bulletins to chapters, treatment centers and volunteer leaders and placed information on our web site. This is only an interim system which wrongly places the burden of notification upon a consumer organization like the NHF, and it highlights the urgent need for the FDA to establish a prompt patient notification system clearly defining the responsibilities of the manufacturer in communicating directly with the consumers and their providers when an adverse event occurs.

In conclusion, more than 2 years after this committee first began to examine blood safety issues, many of the recommendations for a safer blood supply that were part of this committee's blood safety report, the 1995 Institute of Medicine report, and this year's U.S. General Accounting Office report have not been implemented. As a community that has been irreparably harmed by contaminated pooled plasma products and that has been advocating for improvements in collection, testing, manufacturing, viral inactivation, product tracking and recipient notification, we are at a loss to understand why the FDA and manufacturers continue to be reluctant to implement meaningful measures to ensure a safer blood supply.

The bleeding disorder community and others who rely on blood products remain vulnerable to infectious agents entering the U.S. blood supply, but we have no sense that a lesson has been learned from the past.

Thank you, Mr. Chairman.

Mr. SHAYS. Thank you, Mr. Pierce. As you can imagine, we will be following up on some of those recommendations; and, actually, we will be examining some of our own to see if we are still on target.

[The prepared statement of Dr. Pierce follows:]

Thank you for this opportunity to present testimony today on behalf of the National Hemophilia Foundation (NHF). NHF is a national voluntary health organization dedicated to improving the health and welfare of people with hemophilia, von Willebrand's disease, and other bleeding disorders. I am a former president of the Foundation and currently serve as the chair of its Blood Safety Working Group. I am a person with hemophilia and, as a result, have been exposed to many infectious diseases through my use of blood clotting factor. In my professional life, I manage research projects for a biotechnology company.

The National Hemophilia Foundation appreciates the continued efforts of Chairman Shays (R-CT) and this Subcommittee in bringing greater attention to the critical need for safer blood products and a safer blood supply. The Government Reform and Oversight Committee's 1996 report, "Protecting the Nation's Blood Supply for Infectious Agents: The Need for New Standards to Meet New Threats", included critically important recommendations, two of which I will discuss today: reductions in plasma pool size and prompt patient notification.

The hemophilia community continues to be on the front lines of any complication or virus that contaminates the blood supply. While safer blood products are available and today's blood manufacturing processes inactivate HIV, blood and blood products remain susceptible to other pathogens.

The NHF issued 12 medical bulletins in 1996 regarding product investigations, recalls and/or withdrawals and already has issued 12 bulletins this year, including four in July. In addition to the withdrawal of products related to evidence of infectious agents, we are alarmed by the number of recalls this year that have resulted from violations of the Food and Drug Administration's (FDA's) good manufacturing practices where sterility was not maintained, vials were

mishandled, or viral inactivation did not occur at specified temperatures. Even recombinant DNA produced/non-blood based products have been recalled. Although these products are highly unlikely to contain human viruses, potential mold contamination during manufacturing resulted in a recall just last week.

Historically, the hemophilia community has been impacted by a number of viruses through the blood supply. While HIV has been the most devastating, other viruses continue to plague the hemophilia community, including Hepatitis A, Hepatitis B, Hepatitis C, and Parvovirus B19. Strong evidence of the need for a more responsible and responsive blood safety system accumulates as new announcements of blood product recalls are issued, often weeks after the seriousness of a problem has been first detected. Too frequently, individuals in our community find out too late that they just infused themselves or their child with a recalled product.

Plasma Pool Size

In my mind and in that of members of the bleeding disorder community, each recall of product raises serious questions about the safety of the products we use, our exposure to thousands of blood donors, and the impact on supply of needed products. Our community depends on donor deferral, donor testing, and viral inactivation of plasma to protect us from contaminants that may be entering the blood supply. Yet, only one donor can infect an entire plasma pool.

It is important to define this term. Plasma pool refers to the plasma donations that are mixed together for subsequent manufacture into purified coagulation products, immunoglobulins, albumin, and other products. During the manufacturing process, multiple plasma pools may sometimes be mixed

together. The purified product is packaged and distributed in what is referred to as a "lot." Each lot is given a unique number to facilitate tracking. For some products, plasma pool size may sometimes equal lot size. For others, pool size is much smaller, but multiple pools may be added together to make a lot. It is the final lot size that is of concern to the bleeding disorder community as lot size represents the total donors to which blood product recipients are exposed.

NHF was both surprised and troubled to recently learn that there are no upper limits on the number of donors contributing to plasma pools. While manufacturing practices differ, we had been led to believe by FDA and manufacturers that manufacturers were voluntarily observing limits of around 15,000 donors per pool for paid plasma and 60,000 donors per pool for volunteer plasma. We now know that some manufacturers place over 80,000 - 100,000 paid donors into a single lot of product. The absence of any upper limit on pool size places our community at significant risk to emerging infections.

In its own analysis of this issue, the FDA, in an article published last year in the journal *Transfusion*, made the case that larger plasma pools do increase the risk of exposure to, and thus, the risk of transmission of infectious agents, especially to highly susceptible populations such as persons with hemophilia. It is unfortunate, that the authors chose only to conclude that plasma pool size alone would be inadequate in reducing the risk of exposure to persons who repeatedly use blood products. We would have to agree, but reduced pool sizes when combined with other technologies (i.e., improved collection procedures and improved viral inactivation) could greatly diminish current risk exposure probabilities and reduce the amount of product requiring recall when a problem does occur.

Past experience with hepatitis and HIV has demonstrated that not everyone who receives a contaminated lot of product becomes infected, as is assumed in the *Transfusion* article. We need only to look at the spread of HIV to realize that many

patients received multiple exposures to HIV before becoming infected. Exposure to fewer donors would have allowed some individuals to escape infection.

NHF has communicated to FDA its request for limits on the maximum number of donors that can be pooled together in the manufacture of blood products for the following reasons:

- Increased Safety - As emerging infectious viruses threaten or place the blood supply and blood products at risk, reductions in pool size can delay the possibility of widespread transmission.

- Reduction of Exposures - By reducing the total number of donors that a person is exposed to over a period or time, the likelihood of transmission of an infectious disease is minimized. This point is especially relevant to persons with a mild or moderate bleeding disorder (i.e., infrequent users) for whom reductions in pool size may have prevented the transmission of HIV.

- Preserves Product Supply - Under the current situation, identification of a single blood donor with a disease or a risk of a disease can result in the recall of multiple lots of blood product, requiring the return of hundreds or thousands of vials of clotting factor concentrate. Smaller pool sizes and placing donations from a single donor in one lot would result in fewer lots requiring withdrawal or quarantine. Products shortages caused by recalls can be dangerous to the hemophilia community due to the urgency of treating certain bleeds.

Creuztfeldt-Jakob Disease

Recently we have had numerous recalls due to possible Creutzfeldt-Jakob Disease (CJD) contamination. As this Committee knows from previous consideration of this issue, we do not know if CJD can be transmitted by blood products, although it is known to be transmitted by plasma products in experimental animals. Fortunately, no cases of CJD have yet been identified in the bleeding disorders community. However, the number of recalls that have occurred because a donor was later identified as having, or being at risk for, CJD is staggering and has affected the supply of coagulation products in the marketplace. Clearly, if pool size limits were in place, substantially less product would be considered contaminated and require removal from the market because of the identification of a single at-risk donor.

CJD is highly resistant to the viral inactivation techniques employed today to destroy HIV and Hepatitis C. Thus, CJD, along with resistant viruses like Hepatitis A and Parvovirus B19, could be a model for the next potentially deadly pathogen to infect the blood supply. NHF has worked with the National Heart, Lung, and Blood Institute to encourage a study of the scope of vulnerability within the hemophilia community to blood contaminants such as CJD. Research to develop inactivation procedures which destroy these resistant pathogens also is of critical importance to the present and future safety of the blood supply.

Product Recalls/Withdrawals and Patient Notification

We know that recalls occur when the system of donor deferral, donor screening, and viral inactivation, coupled with good manufacturing practices, has broken down. The frequent occurrence of these events (1-2 per month) continues to shake the confidence of consumers and their providers within our community.

As a result of the events of the 1980s where nearly half of all members of our community were infected with HIV, NHF is committed to ensuring that consumers of blood products have information about the products they are using in order to make informed and educated decisions about their treatment. NHF believes this is only possible when they are provided crucial and possibly lifesaving information as soon as possible after FDA investigation of an adverse event begins.

Last year FDA reiterated its position that the blood product manufacturer is responsible for delivering appropriate notification regarding the withdrawal or recall of a specific blood product to the end user of that product, stressing that the FDA has now interpreted the Code of Federal Regulations definition of an "end user" as the actual consumer of the blood product.

Since this announcement, FDA has requested that companies with products in question contact consumer organizations such as NHF, and NHF has issued medical bulletins to its chapters, treatment centers, and volunteer leaders and placed information on our web site. This is only an interim system which wrongly places the burden of notification on NHF and highlights the urgent need for FDA to establish a prompt patient notification system, clearly defining the responsibilities of the manufacturer in communicating directly with consumers and their providers when adverse events occur.

NHF has brought to the attention of FDA the problems that occur when NHF is involved late in the process of a recall or withdrawal of product. We are concerned that FDA has not brought together representatives from our organization, the Centers for Disease Control, the National Institutes of Health, and industry to develop a more complete notification system. This group also could work with FDA to determine the appropriate investigational decisions and objective questions that must be asked to determine when and how notification should occur. The

Food and Drug Administration has in the past relied upon a largely informal system of recalls and withdrawals of blood products. This informality has led to confusion, as reported to this Committee last month by the U.S. General Accounting Office, about the required actions at each level of notification and directly contributes to the lack of effective and prompt communication of product recalls and withdrawals to blood product consumers.

In conclusion, more than two years after this Committee first began to examine blood safety issues, many of the recommendations for a safer blood supply that were part of this Committee's blood safety report, the Institute of Medicine's 1995 report, and the U.S. General Accounting Office report have not been implemented. As a community that has been irreparably harmed by contaminated pooled plasma products and that has been advocating for improvements in collection, testing, manufacturing, viral inactivation, tracking, and recipient notification, we are at a loss to understand why the FDA and manufacturers continue to be reluctant to implement meaningful measures to ensure a safer blood supply and blood products. The bleeding disorder community and others who rely on blood and blood products remain vulnerable to infectious agents entering the U.S. blood supply, but have no sense that a lesson has been learned from the past.

NHF MEDICAL BULLETINS REGARDING PRODUCT WITHDRAWLS SINCE JANUARY 1996

Copies of all medical bulletins can be obtained from HANDI (800/42-HANDI)

Medical Bulletin 237, Chapter Advisory 239, **Investigation of Possible Transmission of Hepatitis A Virus from Alphanine SD**, January 10, 1996

Medical Bulletin 239, Chapter Advisory 241, **Product "Hold" for Specific Lots of Alphanine SD (Lots #CA5410A, CA5412A, CA5413A, CA5421A), Due To Possible Transmission of Hepatitis A Virus**, January 12, 1996

Medical Bulletin 241, Chapter Advisory 243, **Two Companies Announce Voluntary Withdrawal of Specific Lots of Products, Baxter Immune Globulin Intravenous (Gammagard SD) and Albumin (Buminate 24%, Buminate 5%), Bayer Immune Globulin Intravenous (Mannimune N, 10%), Albumin (Plasmanin 25%) and Alpha-1 Proteinase Inhibitor (Prolastin)**, February 15, 1996

Medical Bulletin 242, Chapter Advisory 244, **Alpha Therapeutic Corporation Conducts "Field Exchange" of Lots of Alphanine SD (Lots #CA5410A, CA5412A, CA5413A, CA5421A), Due to Possible Transmission of Hepatitis A Virus**, March 11, 1996

Medical Bulletin 247, Chapter Advisory 249, **One Lot of Alphanate (Lot #AP5015A) Withdrawn Because of Possible Hepatitis A Virus**, May 2, 1996

Medical Bulletin 248, Chapter Advisory 250, **Voluntary Withdrawal of American Red Cross AHF-M and Polygam SD**, June 7, 1996

Medical Bulletin 249, Chapter Advisory 251, **Product Withdrawal of Gammar and Gamulin RH**, June 28, 1996

Medical Bulletin 251, Chapter Advisory 253, **Voluntary Withdrawal of American Red Cross AHF-M**, October 1, 1996

Medical Bulletin 253, Chapter Advisory 255, **Centeon Initiates Recall of One Lot of Monoclate P**, October 7, 1996

Medical Bulletin 254, Chapter Advisory 256, **Speywood Pharmaceutical Recalls Batches of HYATE:C In Europe**, October 9, 1996

Medical Bulletin 255, Chapter Advisory 257, **Centeon Temporarily Suspends Production at Kankakee, Illinois, Manufacturing Facility**, November 1, 1996

Medical Bulletin #266, Chapter Advisory #268, **Update on Suspension of Production at Centeon Plant and Recall of One Batch of Speywood Pharmaceuticals Hyate:C**, December 9, 1996

Medical Advisory #268, Update on Production at Centeon Plant and Continuing Speywood Pharmaceuticals Hyate[illegible] Shortage, February 11, 1997

Medical Advisory #269, Alpha Therapeutics Corporation initiates Quarantine of One Lot of Profilnine SD, February 18, 1997

Medical Advisory #270, Centeon Initiates Voluntary Recall of One Lot of Monoclate-P, February 21, 1997

Medical Advisory #271, Voluntary Withdrawal of American Red Cross AHF-M, Albumin, and Plasma Derivative Products, February 21, 1997

Medical Advisory #272, Centeon Initiates Voluntary Recall of One Lot of Mononine, #P13609, February 28, 1997

Medical Advisory #273, Alpha Therapeutics Announces Lifting of Precautionary Hold on One Lot of Profilnine SD (#LIS6210A), March 5, 1997

Medical Advisory #279, Baxter Recalls 8 lots of Hemofil M, the American Red Cross recalls 1 Lot of AHF-M: Solvent Detergent Treatment Performed at Temperatures Below Cutoff, May 25, 1997

Medical Advisory #280, The American Red Cross Recalls 1 Additional Lot of AHF-M: Solvent-Detergent Treatment Performed at Temperatures Below Cutoff, May 29, 1997

Medical Advisory #281, Baxter Recalls Three (3) Lots of Recombinate due to a Compromise In Sterility At The Manufacturing Plant, July 14, 1997

Medical Advisory #282, American Red Cross Recalls Two (2) Lots of AHF-M Following Two Donor Reports Of Previous Treatment With Human Pituitary Derived Growth Hormone, July 23, 1997

Medical Advisory #283, Additional Product Recalls Due to Risks For Creutzfeldt-Jakob Disease In Donors, July 25, 1997

Mr. SHAYS. Dr. Cunningham.

Dr. CUNNINGHAM-RUNDLES. First, I would like to thank the subcommittee for inviting me to participate in this session on the topic of safety implications of plasma pool sizes in the manufacture of blood products.

My name is Dr. Charlotte Cunningham-Rundles. I am professor of medicine, pediatrics and biochemistry at the Mount Sinai School of Medicine in New York City. I am a member of the Immune Deficiency Foundation's Medical Advisory Committee, and my work for the last 23 years has been in the laboratory study and clinical treatment of primary immunodeficiency diseases.

There are about 50 or more of these diseases and many of these result in frequent and life-threatening infections. Due to these genetic defects, there is an estimated group of more than 20,000 people in the United States—infants, children and an enlarging population of adults—who are not able to make antibodies and who receive regular infusions of a plasma derivative, intravenous gammaglobulin.

The antibodies are complex proteins found in the serum portion of blood, and these proteins are vital for protection against bacterial and viral infections. Since the early 1950's, the standard treatment for immunodeficient patients has been the regular administration of gammaglobulin obtained always from the blood of normal donors. Originally, this was given by intramuscular injection, but since the early 1980's the preferred route of administration has been by intravenous infusion.

Gammaglobulin pools from human blood contain antibodies of a tremendous variety, representing the immune experience of thousands of donors. Patients who don't make their own antibodies are completely dependent upon these infusions which they receive every 3 or 4 weeks, with the expectation of doing so for the remainder of their lives.

I first started to use intravenous formulations of gammaglobulin about 17 years ago, and I think I was one of the first investigators in the United States to use this kind of treatment. Since that time, I have used all of the existing formulations; and I have published a number of articles on the clinical benefits, the biological and immunological effects, and the occasional adverse reactions which might result when using these products.

All the manufacturers fractionate intravenous immunoglobulin from large pools of plasma, using their own proprietary methods and pools of varying sizes. These antibodies, currently at least, cannot be synthesized in the laboratory; and there is very little expectation that molecular techniques will produce any other alternative source of gammaglobulin.

The very reason that the gammaglobulin pools protect our patients is that they contain antibody molecules that protect against the widest possible spectrum of bacteria and viruses. Limiting blood pool donor size will at some point diminish the value of these pools to our patients since the variety of antibodies would necessarily be restricted.

We don't know how small these pools can be and still provide broad antibody protection to these immunodeficient patients. Our point is, before we stipulate donor pool size, we really need to have

this information; and this is the major point that I would like to make today.

An additional point to consider is that patients with immunodeficiency receive immunoglobulin concentrates 12 to 16 times every single year. In our infusion room, where we infuse approximately 100 patients a month, our patients are currently exposed to 10 to 12 lots of intravenous immunoglobulin of their prescribed variety. If the pools are smaller, it is theoretically possible that each of our patients will be exposed to a proportionately larger number of pools. This may reduce or perhaps eliminate the benefits of reducing the pool size.

These patients are frequent and lifelong users, and we believe it becomes imperative that they have representation on the Health and Human Services Advisory Committee on Blood Safety and Availability and the FDA's Blood Product Advisory Committee. Decisions made regarding manufacturing processes, safety and availability must incorporate the unique concerns of the immune-deficient patient population.

Since the introduction of intravenous immunoglobulin, our patients can look forward to a normal life span. However, adverse reactions have occurred with the administration of immunoglobulin; and in some cases these have forever changed and in a few instances ended the lives of our patients.

Most recently, our patients have experienced an outbreak of hepatitis C due to the use of intravenous immunoglobulin products. People with genetic immunodeficiency appear more likely to develop fulminant viral hepatitis and liver failure for reasons that we don't understand.

As far as I am aware, there is no information available on the total number of cases which have occurred; we think that a national registry compiling the natural history of this disease in this patient group is needed. Some information about the patient's response to interferon, if used, results of liver transplantation, if performed, would be a very valuable resource for physicians who are still dealing with the aftermath of this outbreak.

The third issue I would like to touch upon is the means of notification of product withdrawal and notification. I have received a number of notices of recalled or withdrawn products of gammaglobulin in the past year or so. These notices have come from a number of different sources, including the Immune Deficiency Foundation, manufacturers, the National Organization of Rare Diseases and, in some cases, home care companies.

On the other hand, I have never had a notification from my own hospital pharmacy, and I have never had one from the wholesale distributors that I use. There may have been recalls or withdrawals for which I have received no notification.

Since mine is a very large clinical practice dealing specifically with primary immunodeficiency, I can only imagine the difficulty amongst internists and pediatricians who only see a few patients in their practice annually.

Because of the lack of an organized notification process, it is really likely that primary immunodeficient patients will receive an intravenous immunoglobulin infusion this week from recently withdrawn lots.

As a final note on the issues of notification, the Immune Deficiency Foundation, for whom I serve as a voluntary medical advisor, reports that it is common practice for some manufacturers to advise them of recalls or withdrawals, but other manufacturers do not do that. It is also my understanding that the FDA does not routinely inform the IDF of recalls or withdrawals either. Keep in mind that, at the present time, the IDF organization and treating physicians like myself are the only direct links to patients.

To summarize, I would like to leave the subcommittee with the following recommendations or suggestions.

No. 1, the FDA, NIH or CDC should initiate a systematic study into the relationship of plasma pool sizes and the antibody content of intravenous immunoglobulin preparations. I think industry could be a very active collaborator in such a study.

No. 2, the FDA, NIH and CDC should establish sufficient lookback and health surveillance programs within the immunodeficient population who do use intravenous immunoglobulin to assess past exposures and current and future risks. I am talking especially about hepatitis C.

The FDA and industry must rapidly address the issue of effective physician and patient notification of recalls and withdrawals. The Immune Deficiency Foundation will assist or advise in any way possible.

We think it absolutely imperative that representatives of the primary immunodeficient patient population, whether they are medical professionals or patients, be appointed to the existing blood advisory panels so that they may assist regulators and industry in avoiding mistakes.

Thank you very much for the opportunity to present this information.

[The prepared statement of Dr. Cunningham-Rundles follows:]

IMMUNE DEFICIENCY FOUNDATION

The National Organization Devoted To Research And Education For The Primary Immune Deficiency Diseases.

Charlotte Cunningham-Rundles, M.D. Ph.D.

Dr. Cunningham-Rundles is currently Professor, Department of Medicine, Pediatrics, Biochemistry at Mount Sinai School of Medicine. Dr. Cunningham-Rundles resides in New York City. She received her M.D. from Columbia University and her Ph.D. From New York University Medical Center. Dr. Cunningham-Rundles is widely recognized as a leading expert in the field of primary immunodeficiencies and the use of intravenous immunoglobulins. Dr. Cunningham-Rundles participated in the 1990 NIH, Consensus Conference on the uses of intravenous immunglobulin in the treatment of primary immunodeficiency diseases. She participates in visiting professorships, and professional society meetings as an educator in the treatment of primary immunodeficiency diseases. Dr. Cunningham-Rundles is a member of the Immune Deficiency Foundation's Medical Advisory Committee.

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I would like to thank this Subcommittee for inviting me to participate in this session on the topic of safety implications of plasma pool sizes in the manufacture of fractionated blood products. My name is Charlotte Cunningham-Rundles, and I am a professor of Medicine, Pediatrics and Biochemistry at the Mount Sinai School of Medicine in New York City. I hold MD and PhD degrees. I am also a member of the Immune Deficiency Foundation's Medical Advisory Committee. My interests have been in the laboratory study and clinical treatment of primary immunodeficiency diseases. There are about 50 or more of these diseases; many of these result in frequent and life-threatening infections. Due to these genetic defects, there are an estimated group of more than 20,000 people in the United States, including infants, children and an enlarging population of adults , who are not able to make antibodies, and who receive regular infusions of a plasma derivative, intravenous gammaglobulin.

Antibodies are complex proteins found in the serum portion of the blood called gammaglobulin or immunoglobulin; these proteins are vital for protection against bacterial, viral, and other infections. Since the early 1950's, the standard treatment for these immunodeficient patients had been regular administration of gammaglobulin obtained from the blood of normal donors. First given by intramuscular injection, since the mid 1980's the preferred route of administration had been by intravenous infusion. Gammaglobulin pools from human blood contain antibodies of tremendous variety representing the immune experience of thousands of donors. Patients who do not make their own antibodies are completely dependent upon these infusions, which they receive every three or four weeks, with the expectation of doing so for the remainder of their lives.

I first started to use intravenous formulations of gammaglobulin about 17 years ago, and was thus one of the first investigators in the United States to use these substances. Since then I have used all the existing formulations, and have published a number of articles on the clinical benefits of this treatment, the biological and immunological effects of these molecules, and the adverse reactions which may result when using these biological products. All manufacturers fractionate intravenous immunoglobulin from large pools of human plasma, using their own proprietary methods and pools of varying sizes. These antibodies cannot be synthesized in a factory and there is little expectation that recombinant techniques will produce alternative sources of gammaglobulin. The very reason that gammaglobulin pools protect our patients is that they contain antibody molecules that protect against the widest possible spectrum of bacteria and viruses. Limiting blood donor pool size will, at some point, diminish the value of these pools to our patients, since the variety of antibodies would necessarily be restricted. We do not know how small these pools can be and still provide broad antibody protection to the immunodeficient patient. Before we stipulate donor pool size, we must have this information. This is the first point that I would like to make today.

An additional point to consider is that patients with immunodeficiency receive immunoglobulin concentrates 12 to 16 times a year. In our infusion room, where we infuse about 100 patients per month, we have determined that our patients are currently exposed to 10 to 12 lots of their prescribed immunoglobulin product per year. If pool sizes are smaller, I assume that patients could be exposed to a proportionally larger number of lots. This would reduce or eliminate the benefits of reducing the pool size.

Because these patients are frequent and lifelong users it becomes imperative that they have representation on the Health and Human Services, Advisory Committee on Blood Safety and

Availability and the FDA's Blood Product Advisory Committee. Decisions made regarding the manufacturing process, safety and availability need to incorporate the unique concerns of the immune deficient patient population.

In the past 45 years the immunodeficient population has benefited, as well as experienced some adverse consequences, through the use of these blood products. Since the introduction of intravenous immunoglobulin, our patients can look forward to a normal, or near normal life span; however, adverse events associated with the administration of immunoglobulin have occurred, and have forever changed or ended the lives of some patients. Most recently our patients have experienced an outbreak of hepatitis C due to the use of two intravenous immunoglobulin products. People with genetic immunodeficiency appear more likely to develop fulminant viral hepatitis, and liver failure. As far as I am aware there is no information available on the total number of cases which have occurred. A national registry compiling the natural history of this disease in this patient group, predictors of patients' response to interferon, and results of liver transplantation, if performed, would be a most valuable resource for physicians and scientists who are still dealing with the aftermath of this outbreak.

The third issue that I would like to touch upon is the means of notification of product recall and withdrawal. I have received a number of notices of recalled or withdrawn lots of intravenous immunoglobulin in the past year or so. In preparation for this testimony I reviewed their various origins. These notices were received in my office from a variety of sources, which include the Immune Deficiency Foundation, manufacturers, the National Organization of Rare Diseases, and home health care companies. I have never had notification from my own hospital pharmacy, nor from the wholesale distributors I use.

Although time has not permitted me to conduct a through review since this subcommittee's invitation last week, it is reasonable to assume given the inconsistent nature of the current notification system, that there may have been recalls or withdrawals in the past for which I have received no notification whatsoever. Since mine is a large clinical practice specializing in primary immunodeficiencies one can only imagine the situation among internists, pediatricians and others who see only a few primary immunodeficient patients in their practices annually. Because of IGIV product in the pipeline and because of a lack of an organized notification process, it is altogether likely that primary immunodeficient patients will receive IGIV infusions this week from recently withdrawn lots.

As a final note on the issue of notification, the Immune Deficiency Foundation for whom I serve as a volunteer Medical Advisor reports that while it is common practice for some manufacturers to advise them of recalls and withdrawals other manufacturers do not. Additionally, it is my understanding from conversations with IDF staff that FDA does not routinely inform IDF of recalls or withdrawals. Keep in mind that the IDF organization and treating physicians like myself are the only direct links to patients.

To summarize I would like to leave the Subcommittee with the following recommendations or suggestions:

1. The FDA, NIH, and or CDC should initiate a systematic study into the relationship of plasma pool sizes and the antibody content of IGIV preparations. Industry must be an active collaborator in such a study. The Immune Deficiency Foundation is willing to assist.

2. The FDA, NIH, and CDC should establish sufficient look-back and health surveillance programs within the primary immunodeficient population using IVIG, a significant consumer population, to assess past exposures, viable treatments, and current and future risks.

3. FDA and industry must rapidly address the issue of effective physician and patient notification of recalls and withdrawals. The Immune Deficiency Foundation will assist and advise in any way possible.

4. It is absolutely imperative that representatives of the primary immunodeficient population, be they medical professionals or patients, be appointed to the existing blood advisory panels so that they may assist regulators and industry in avoiding tragic mistakes.

Mr. SHAYS. I thank all three of you.

Your testimony is confirmation why I like the consumers basically to go first. There is a kind of policy that has been there so long that the department heads get to address Congress first; and if we don't allow that to happen, it is considered a slight of the legislative on the executive branch. But you have raised some questions that I wish I had asked our first panel.

Mr. Towns, do you want to begin?

Mr. TOWNS. Thank you, Mr. Chairman.

Dr. Pierce, can you tell us about your group's feelings about the CDC's involvement in funding of hemophiliac treatment centers?

Dr. PIERCE. Yes. For a number of years now, probably close to 8 or 10, the CDC has been actively funding a number of risk reduction programs in the hemophilia community, both at the level of consumer-based chapters as well as at our hemophilia treatment centers.

Initially, they were designed to decrease the risk of transmission of HIV, which came via the plasma pool, to uninfected spouses and sexual partners and children. So that was the initial impetus, was to prevent the subsequent transmission of additional infections.

The emphasis has changed in more recent years with an emphasis on the prevention of other complications of hemophilia as well, including joint disease, other infectious diseases such as hepatitis C, which, if HIV were not in the community, hepatitis C would be recognized as a major killer of individuals with bleeding disorders.

So the CDC has taken a more active role in looking at those complications as well.

Mr. TOWNS. Thank you very much. I was getting ready to say that light did not give me 5 minutes.

We talked about notification. CDC has conducted a public service campaign to notify people of possible exposure to hepatitis C. Would any of the witnesses like to comment on that campaign? Any of you?

Dr. PIERCE. I am not sure, from our perspective, we can comment on it. In the hemophilia community, they have worked closely with our treatment centers, so virtually everybody in our community has been tested for hepatitis C infection; and it is being followed medically at this point.

Mr. TOWNS. So you would not be.

Dr. CUNNINGHAM-RUNDLES. In the immune-deficient patient population, many studies were done regarding the incidence of that infection. I think everyone was feeling quite complacent until approximately 1993 and early 1994, when it was discovered that many patients were suddenly becoming infected with a product they had considered safe for some 10 years prior to that.

After that, a rather intensive sort of surveillance has been undertaken by most physicians dealing with patients who have received this form of gammaglobulin using the PCR tests. So I think we now have a pretty good means of knowing who is infected and who is not.

The only difficulty is I think that not all patients know exactly which gammaglobulin they may have received. They don't know that they are at risk; and, in fact, the infection could perhaps be dormant and not tested for.

Mr. TOWNS. I am looking for an answer on notification and withdrawal: what can we do on this side? Just change roles for a moment. What can we do on this side to enhance that in terms of that? I am concerned about the fact that if there is a problem and then there is no real recall or if nobody pays any attention to it or they put it in small print and nobody reads it, what can we do on this side to make certain that, when something like this occurs, that there is vigorous action taken on the part of the manufacturers and everybody involved? What can we do?

Dr. PIERCE. Well, in your role of oversight of the FDA, I think the FDA needs to make sure that the manufacturers are accountable for getting notification out about product recalls rapidly, with direct consumer and physician notification. That is the subject of interpretation in the Code of Federal Regulations.

Industry has suggested that there may be new laws that are required. The FDA, at least as far as I had heard in the past, has suggested that is not the case. They believe they have the ability to enforce that. You would have to ask them for more specifics.

But we seem to be at a stalemate here, where nothing is occurring that really will officially get that information out to people.

Mr. TOWNS. I think what I am saying is that maybe we should look at some legislation that might bring about some specifics with some penalties and all that involved? I don't know. I think this is why we have these hearings, to try to get information. I see there is a problem, and I think something needs to be done, but I am not sure what.

Dr. PIERCE. Well, someone needs to figure out if the FDA already has the authority to do it; and, if they do, then it needs to be enforced. If they don't, then we need legislation that gives them that authority.

Mr. TOWNS. It is my understanding to some extent—and I could be wrong—that the size of the plasma pool is a business decision which is guided by economics and necessity. I understand in order to produce some plasma products the producer needs a certain pool size. I understand that.

Would any of the witnesses care to comment on the economics versus the public health concerns? I get the feeling there are some economics tied into this. I could be wrong.

Dr. PIERCE. You know, the economics work both ways. On the one hand, if you have a smaller manufacturing process or smaller number of pools put together to form a lot, yes, that will cost more, that may decrease the amount of product; and we will probably hear about that from industry this afternoon.

On the other hand, the number of recalls that are occurring has already had a significant economic impact as well as a significant impact on supply. So there is a balance there.

When you are talking about pool sizes or lot sizes, however we want to define it, that are in the hundreds of thousands, I will go back to the first panel and say, common sense tells you that is just way too high from a public health perspective, for all the reasons that have been outlined earlier today.

Mr. TOWNS. Any other comments on that?

Dr. CUNNINGHAM-RUNDLES. Well, I think we don't know how small the pool size should be either. I see the number 1,000 ban-

died around from place to place. I don't know where that number 1,000 came from. I don't even know if it is accurate. So even the lower limit I think is something which is subject to challenge at this point.

I know that for the immune-deficient patient population, there is a rare central nervous system disease called echo virus; and there have been situations, especially I would say in the last 10 years—not commonly, but it will occur—where physicians are having to test various lots of gammaglobulin to make sure that they have enough antibody present in a given preparation to treat their patient. That says to me that perhaps those lots don't contain enough broad spectrum of antibody.

So the lower limit I think is another issue that we don't talk about very much, but I suspect that may be as important.

Mr. TOWNS. That is a good point.

Let me just sort of ask a last question here. There are a few bills in the House that would establish a compensation fund for people who have contracted HIV as a result of exposure to blood or blood products. I must admit I am a cosponsor of one of them.

I would like to know the thoughts of anyone on the panel who would care to comment on the compensation idea. Give me your views and feelings around this whole compensation concept.

Ms. CROOKER. Compensation—basically, the hemophiliacs have been the watchdogs, they have been the canaries or whatever you want to call us, for the blood supply here in the United States and in the world. Anything that goes wrong in the blood supply, they come down with it first. So, you know, if there is no problems with the hemophiliacs, then the blood supply is fairly safe.

You pay your guards, you pay—you even pay for your canary, those that they take down into the mines. We should get some compensation in this sense if for no other reason. Besides, it was an FDA-approved product.

Dr. CUNNINGHAM-RUNDLES. I suppose I feel similarly.

With regards to the patients with hepatitis C, with primary immune-deficiency disease, the situation is no different. That virus, in fact, was in certain preparations for reasons that we don't completely understand, but the patients certainly weren't at fault.

Dr. PIERCE. The hemophilia community, as Ms. Crooker said, has been on the front lines. There has been a failure of the system in the 1980's that resulted in this devastating infection, and we are working very hard to see that the Ricky Ray bill is passed because of that.

Mr. TOWNS. I thank all the members of the panel.

Mr. Chairman, let me thank you. It is so important that we are able to spend the time to get information, because it is a very serious issue, and we need to know as much about it as possible. Thank you for your generosity.

Mr. SHAYS. I thank you.

Mr. Pierce was thanking me for being involved in this effort. Mr. Pierce, I think you know that Mr. Towns has really been an equal partner in this effort, as have some of the other members—Dr. Pierce, I am sorry.

Dr. Pierce, I am taught to think of the hemophiliac community as kind of the canary in the coal mine, so I know you are right in

the front lines in this whole issue. I really do wish that we had gone with this panel first, because we didn't deal with any of the recall issue.

I would like each of you to tell me in your mind how the system works. Ms. Crooker, you lost your precious grandson, and then 2 years after his death you were notified that one of the pools that he had used had a contaminated source.

Ms. CROOKER. No. It was 5 months before his death.

Mr. SHAYS. I am sorry.

Ms. CROOKER. I had got information that 2 years before that he had received a lot of factor contaminated with possible CJD, but it was 2 years after his death to get the results that he had not died of CJD or CJD was not present in his system.

Mr. SHAYS. They determined later it was not.

Ms. CROOKER. It was not.

Mr. SHAYS. So what would be your general point? What should I learn from your experience?

Ms. CROOKER. Well, I know the treatment center in New Jersey is very fast, when they get recalls, of notifying the families of the community about recalls, but it seems like that there is such a delay between their notification from industry. So I think the industry has to be within a certain time limit or the time limit must be shortened between their notification of the general public.

Mr. SHAYS. In the course of the 12 years with your grandson, had you experienced recalls, notices of recalls and so on?

Ms. CROOKER. There was a lot of recall notices when he was younger with the HIV, and they came very slow also. In fact, a lot of times my recall notices came well after I had used up all the product.

Mr. SHAYS. So when that happened, for instance in HIV notification, would you go and have your grandson tested for HIV? How does someone respond when you get a notice like that?

Ms. CROOKER. Roger was at a time where he just received heat-treated factor——

Mr. SHAYS. Right.

Ms. CROOKER [continuing]. So it was not until some time around 1987 where he received a recall factor from Armor; and at that time they said there was no need because this was not his lot numbers, because he had only received heat treated. It came to be that, because of the second recall factor, that he received about a year later, I believe it was, it was a group that he again had received; and shortly after that they finally did the testing.

Mr. SHAYS. When you get a notice, is it something that you just kind of are blase about?

Ms. CROOKER. No. We are very aware of the notice. We check the lot numbers we have on record.

Mr. SHAYS. I see. Dr. Pierce.

Dr. PIERCE. I would like to make three points regarding recall and notification.

The first is the sheer number of recalls that have occurred as a result of a failure of good manufacturing practices. I am not aware—there may be some, but I am not aware of any other FDA-approved drugs that have had this kind of history over the past year. That has caused a tremendous amount of anxiety in the com-

munity, because we have got these products at home, we use them at home in our refrigerators, and we are completely dependent.

Mr. SHAYS. So when you get notification like that and you are looking at a lot size, you are going down, you are going to get this lot, this medicine, the plasma and so on, looking at it and holding your breath to determine if this is one that is recalled?

Dr. PIERCE. Yes, to a large degree. You wonder, when you use the material, has this been recalled, and I'm just not aware of it yet; is this product under investigation, and I'm not aware of that yet.

Mr. SHAYS. When you get a recall—I'm just trying to anticipate—when you get a recall, you immediately check?

Dr. PIERCE. Yes. There is no question. But if you've—if you get a recall notice for a particular product, you look at the lot numbers on that product and make sure you don't have that product.

Mr. SHAYS. OK.

Dr. PIERCE. The other point to make on the notification is that it is a very haphazard. It doesn't by—industry is not accountable to take notification down to the end user, which the FDA has now specified is the consumer of the product, the person who is infusing that product into their veins. And without that, then you are left uncertain as to whether or not the end user in all cases has gotten the information.

Mr. SHAYS. I wonder if the economics would require them to try to contact the end user. I don't know how feasible it is, if there would be an added incentive to have a smaller lot size.

Dr. PIERCE. Well, if you're able to go up the system by writing a prescription, sending that in, getting product from the manufacturer, you should be able to go down the system following that same pathway and make sure that you contact the person for whom that prescription was written for if there's a problem.

Mr. SHAYS. Dr. Cunningham, do you want to explain how the recall system works from your standpoint?

Dr. CUNNINGHAM-RUNDLES. From my standpoint, we buy very large amounts of gammaglobulin at a time, and we stock it in our infusion area, and when we get a recall, we check to see whether we have any cases that match that lot. In most cases, the recall comes quite a bit after the lots have already been used, and that's partly because CJD recalls, for example, often happen retrospectively. The donor was later found to have come from a family in which a case was reported, so it's far too late. It might have been even several years previously. So that's the first issue.

The second thing is, since we buy it from large distributors, they have no idea which lot which patient got. So it becomes, I think, equally impossible for a manufacturer to know what lot an individual patient got given.

The other issue is that I think a good number of physicians in the United States don't write down lot numbers at all, and so that's another issue that I think is important. The only way I can think of to do it is to put a box top into the bag of gammaglobulin and have it returned to the manufacturer, and that's obviously incredibly labor-intensive. It's almost like a coupon in a box of Cheerios. And that would be very impractical perhaps.

But the net—the end user is the only one who really ends up needing the information, but that turns out to be filtered with a good number of layers between that individual and the manufacturer.

Mr. SHAYS. Is the—I want each of you to tell me your position on lot size and why. You've done it in your testimony, you in particular, Dr. Pierce, but I want you to say what you would like to see as a consumer. It is clear from our committee's standpoint that we have thought that the general lot size was in the tens of thousands, not in the hundred thousands, the pool size—I don't mean the lot size, I'm sorry, in the pool size was in the tens of thousands, not in the hundred thousands. Does it make a difference to you? And if so, explain to me why it makes a difference.

Ms. CROOKER. Lot size to me is important just from the standpoint in the sense that the more there is, the more chances of contamination. But I think a standard has to be made. This way, in a standard all across, you would know that in that standard there was a high present and there was a low present. If there was a variation in that, then you know there was something wrong in that lot size.

Mr. SHAYS. So you want standardization and limit——

Ms. CROOKER. Limitation of size, less exposure.

Mr. SHAYS. OK. Dr. Pierce.

Dr. PIERCE. I think the manufacturers have been allowing us to confuse pool sizes and lot sizes for the last couple of years. And we've been led to believe that the pool size, which we've translated into the lot size, is about 15,000 donors for source plasma. They have allowed that to occur. They have told us that, knowing full well that they mix these pools together, which we weren't aware of, and that their lot sizes are, in fact, much, much higher. So I'm coming at this from the perspective of feeling like I've been deceived by a number of individuals on this issue.

Mr. SHAYS. I mean—and you follow this issue very closely. You're not a casual participant in this process, you're someone who has spent a lot of time and—and when did you become aware of the difference between pool size and lot size?

Dr. PIERCE. I first became aware of it November 1996 when I was told by an individual at the FDA that there was a very real distinction and that pools were mixed together to form lots. I later queried industry by sending a letter to all of the manufacturers, asking them what the maximum number of units were, maximum number of donors were in their lots over the past 3 years and what the average number was. That was when I first got information from most of the manufacturers telling me that they were in the high, high—well they were in the hundred thousand range, up to the hundred thousand range.

Mr. SHAYS. Well, it's just like when this committee had a hearing on Gulf war illnesses, and we asked about chemical exposure, and we were told there was no offensive use of chemicals, therefore there was no chemical exposure when there was defensive exposure to chemicals; in other words, we blew up the depots and so on. It's just—you feel like you're in a war game.

Dr. PIERCE. Exactly. You are playing 64 questions, and you just have to figure out what the right question is to ask. And it's an iterative process.

Mr. SHAYS. OK. Dr. Cunningham.

Dr. CUNNINGHAM-RUNDLES. I don't think I would be able to say——

Mr. SHAYS. The question is——

Dr. CUNNINGHAM-RUNDLES [continuing]. With any scientific certainty how big or how small the pool size would be. I know that the major impact right now for large pool size for us is a chronic shortage of individual products of gammaglobulin. So the patient doesn't know if they're going to be able to get their infusion. The home care company will cancel repeatedly.

Mr. SHAYS. Is the larger the pool size——

Dr. CUNNINGHAM-RUNDLES. It's more likely the recall will affect greater numbers of bottles, and therefore we'll be temporarily short-stocked on many different products. And that, to me, is the single daily most annoying headache about the large pool size.

Mr. SHAYS. OK.

Dr. CUNNINGHAM-RUNDLES. Yes, there is the very strong issue of infectivity, and we cannot look past that. It's also very important to say that we also think there's a lower limit that should be adopted, although the scientific evidence for that is not in. I think we should gather it.

Mr. SHAYS. OK. Is there anything else that the three of you would like to say before we get to our next panel?

Dr. CUNNINGHAM-RUNDLES. There's one more point I would like to make. It hasn't been brought up in this particular committee, but this has to do with the growing use of intravenous immunoglobulin for autoimmune diseases. It's not something which is part of my role at the Immune Deficiency Foundation, but as a clinical immunologist. You should be aware that the major use right now is for these diseases, and not the ones that I've discussed or we've discussed today. We don't know why it works in those ailments. Many people have suspected that it might be due to the presence of illusive secondary antibodies called anti-idiotypic antibodies. If this is the case, then it could be that we would be reducing pool size and eliminating value which we inadvertently got by large pool size. So we must somewhere in our thinking process recall that is another usage and perhaps should be thought about as well.

Mr. SHAYS. Thank you very much. I appreciate all three of you being here. Thank you.

Dr. PIERCE. Thank you.

Mr. SHAYS. We're going to go to our third and final panel and ask for Dr. Richard Davey, Mr. Robert Reilly, Mr. Michael Fournel, Dr. Ed Gomperts, Dr. Fred Feldman and Ms. Sue Preston.

I thank all of our six witnesses for being here. And as you know, we need to swear you in, so if I could ask you to stand and raise your right hands.

[Witnesses sworn.]

Mr. SHAYS. Thank you very much. I appreciate all of you being here. This is a very important hearing, very important issue, and we don't pretend to have the answers. We're wrestling with this

issue, obviously, as you can imagine, and want to make constructive contribution. So it's nice to have you here.

I think you can imagine, with six witnesses, I'm going to be a little stricter with time. And I'm making an assumption that you have some—in some cases will coordinate your testimony to some measure that—OK. I'm looking at some question marks here. The bottom line is, if you feel that the issue has been covered by someone else, you can just kind of say, "ditto." But I am going to be strict on the 5 minutes given that we have six witnesses.

And we'll start with Dr. Davey, and we'll go as I called you. Let me just say you're actually sitting the way I called you.

May I just ask beforehand, it just helps me sort out—we have three manufacturers—we have four manufacturers plus the industry representative, and that's you, Mr. Reilly.

Is it fair to say that the testimony—have you all shared your testimony? I mean, do you all know what the others are saying? This isn't antitrust.

Dr. DAVEY. No, we haven't.

Mr. SHAYS. But I'm making an assumption that your testimony, Mr. Davey, will be slightly different than the other testimony. I'm going to give you a little more flexibility with the 5-minute rule. I'm just going to say that.

And, Dr. Reilly, I'll give you a little more—Mr. Reilly, I'm sorry—a little more flexibility with the 5 minute rule, giving you're representing the entire group. But I will be strict with the four of you, if that's all right. That's the way I will proceed. OK.

So, if you didn't get to cover it in your testimony, we'll try to get it in your questions.

So we'll start with you, Dr. Davey.

STATEMENTS OF RICHARD DAVEY, M.D., CHIEF MEDICAL OFFICER, AMERICAN RED CROSS; ROBERT REILLY, EXECUTIVE DIRECTOR, INTERNATIONAL PLASMA PRODUCTS INDUSTRY ASSOCIATION; MICHAEL FOURNEL, VICE PRESIDENT, BIOLOGICALS DIVISION, BAYER CORP.; ED GOMPERTS, M.D., VICE PRESIDENT, MEDICAL AFFAIRS & CLINICAL DEVELOPMENT, BAXTER HEALTHCARE CORP.; FRED FELDMAN, Ph.D., VICE PRESIDENT, CENTEON CORP.; AND M. SUE PRESTON, VICE PRESIDENT, QUALITY & REGULATORY AFFAIRS, ALPHA THERAPEUTIC CORP.

Dr. DAVEY. Thank you very much, Mr. Chairman, Representative Towns, and members of the subcommittee for inviting me to speak about this important issue of plasma derivative safety. I'm Dr. Richard J. Davey. I'm a board certified hematologist, and I'm the chief medical officer of the American Red Cross Biomedical Services, and Alternate Responsible Head for FDA License 190 under which our blood services program operates.

The American Red Cross is the largest not-for-profit provider of blood services in the United States, collecting almost 6 million units of whole blood from volunteer donors annually, or about 45 percent of the Nation's blood supply. Blood collected for transfusion is made into specific components such as red blood cells, platelets, and plasma, which Red Cross distributes to over 3,000 hospitals in the United States.

In addition to those components, approximately 1 million liters of plasma recovered from our volunteer blood donor units are annually processed or fractionated into plasma derivatives. Approximately 800,000 liters are fractionated at Baxter Healthcare's Hyland Division under that company's FDA license, and approximately 200,000 liters are fractionated by the Swiss Red Cross under its FDA license. These plasma derivative products are distributed under the Red Cross label to hospitals, hemophilia treatment centers, and other intermediaries. The Red Cross itself does not fractionate plasma.

Plasma derivatives manufactured from Red Cross include Factor VIII Concentrate, albumin, and immune globulins. Red Cross plasma derivatives account for approximately 15 to 20 percent of the Nation's supply and are produced solely from voluntary nonremunerated donations.

I've been asked to comment on the role of plasma pool size in relation to plasma derivative safety and to outline new safety initiatives which will ensure that Red Cross plasma products will continue to be manufactured by state-of-the-art methods.

Before doing so, it is necessary to distinguish between recovered and source plasma. Red Cross plasma derivatives are made from voluntary whole blood donations. Plasma obtained when whole blood is divided into components is called recovered plasma. In contrast, plasma derivatives made by commercial companies are manufactured principally from plasma obtained by a procedure called plasmapheresis. And plasma obtained by plasmapheresis is called source plasma.

The amount of recovered plasma from a unit of whole blood averages 250 milliliters. The amount of source plasma obtained by plasmapheresis averages about 700 milliliters. Therefore, an initial pool of recovered plasma contains plasma from more than two to three times the number of donations as the same size pool made exclusively from source plasma.

The Red Cross has taken several steps to reduce the number of donations in pools of recovered plasma. In early 1996, we directed Baxter to initiate and validate processes to ensure that Red Cross, AHF-M and IVIg, or Polygam S/D, are derived from pools containing approximately 16,000 liters, or between 54,000 and 60,000 donations.

Since mid-1996, the vast majority of Red Cross AHF-M and IVIg have been derived from pools containing fewer than 60,000 donations. Importantly, this process ensures that the albumin used to stabilize AHF-M and IVIg is also derived from the same pool; in other words, material from different pools is not mixed together. Efforts will continue over the next year to reduce pool size to similar levels for the production of albumin that's intended for transfusion.

These efforts to limit the number of donors in plasma pools will continue. Our commitment to safety is demonstrated by our record. Over 1 billion units of American Red Cross AHF-M have been infused since the latest generation of AHF-M was introduced in 1988, with no reported cases of viral transmission.

Pool size is only one of the elements to consider in improving the safety of plasma derivatives. The Red Cross is actively exploring

new methods to inactivate or remove potentially transmissible agents from blood and plasma. These methods include gamma irradiation, iodine treatment, and the use of high-efficiency filters. These techniques can be effective against both known and newly emergent threats to plasma derivative safety.

Within the next year, the Red Cross will also implement a highly sensitive testing technology called polymerase chain reaction, or PCR, to detect early evidence of infectious virus in plasma to be processed into derivatives. Preliminary studies suggests that PCR testing may prevent the transfusion of several hundred blood components each year that may be infectious for hepatitis C.

I've also been asked to address the evidence regarding the potential for transmission of Creutzfeldt-Jakob disease, or CJD, through the blood supply and to review Red Cross research in this area.

The Red Cross takes all potential threats to blood safety and plasma safety very seriously, and we've moved aggressively to expand the body of scientific information related to CJD. We have several research studies underway at our Jerome Holland Laboratory and, as you've heard this morning, in collaboration with Dr. Paul Brown at the NIH, and also with Dr. Robert Rohwer at the Veterans' Administration.

The Red Cross has committed over $1 million in research studying possible links between CJD and transfusion, probably more than any other private organization. We've also taken steps to reduce the likelihood that plasma from a donor subsequently diagnosed with CJD is included in pools for fractionation.

CJD is a disease, as you've heard, of older people, with a mean age of incidence, to my understanding, of 67 years old. The Red Cross only uses plasma from donors 59 years old or younger for fractionation, thus eliminating the age group at greatest risk for CJD from plasma pools. Plasma from older donors continues to be used beneficially as single donor products.

The Red Cross is also conducting a CJD lookback study with the CDC, as you have heard this morning from Dr. Satcher. That study is under the direction of Marion Sullivan at our Red Cross Holland Laboratory. She studied 179 recipients of blood transfusions from donors subsequently diagnosed with CJD. These recipients have been followed for up to 25 years following transfusion. None of the recipients has died of CJD, and none has shown any sign of the illness. These data are encouraging. Until there is further convincing evidence of nontransmissibility, however, the Red Cross will continue to quickly withdraw plasma derivatives following receipt of postdonation information from a donor or a donor's family about a risk of CJD.

In conclusion, Mr. Chairman, the American Red Cross is committed to providing an adequate supply of blood components and plasma derivatives to meet the highest standard of safety. Red Cross plasma derivatives are proven to be both safe and effective. We've taken steps to ensure this safety by reducing the number of volunteer recovered plasma donations in pools for fractionation and by eliminating plasma from donors in age brackets most likely to be affected by CJD. These steps are part of a larger program to improve safety by an aggressive quality assurance program, focused research programs, and improved donor screening and testing. The

Red Cross takes the issue of blood safety very seriously. We're proud of our record and of our tradition of serving the American people.

Thank you, Mr. Chairman.

Mr. SHAYS. Thank you, Dr. Davey.

[The prepared statement of Dr. Davey follows:]

Thank you very much Mr. Chairman, Representative Towns, and members of the Subcommittee, for inviting me to speak with you about the important issue of plasma derivative safety. As I will be presenting only a portion of my written statement today, I ask that the complete statement be made part of the record. I am Dr. Richard J. Davey, Chief Medical Officer of the American Red Cross Biomedical Services and Alternate Responsible Head for FDA License 190 under which our blood services program operates. I also chair the Research and Development Committee of the Red Cross, and work closely with Red Cross physicians and the scientists at our Jerome Holland Laboratories on a variety of issues relating to the safety of blood components and plasma derivatives. Before joining the Red Cross I was with the National Institutes of Health for 19 years, most recently as Chief of Laboratory Services with the Department of Transfusion Medicine. I am a board certified hematologist and am an Associate Professor of Medicine at Georgetown University Hospital. I have also been a medical officer with the Global Blood Safety Initiative of the World Health Organization.

The American Red Cross is the largest not-for-profit provider of blood services in the United States, collecting almost 6 million units of whole blood from volunteer donors annually, or about 45% of the nation's blood supply. Blood collected for transfusion is made into specific components such as red blood cells, platelets and plasma, which Red Cross distributes to over 3,000 hospitals in the United States.

In addition to those components, approximately 1,000,000 liters of plasma recovered from our volunteer blood donor units are annually processed, or fractionated, into plasma derivatives. Approximately 800,000 liters are fractionated at Baxter Healthcare's Hyland Division under that company's FDA license, and approximately 200,000 liters are fractionated by the Swiss Red Cross under its FDA license. These plasma derivative products are distributed under the Red Cross label to hospitals, hemophilia treatment centers, and other intermediaries. The Red Cross does not itself fractionate plasma.

Plasma derivatives manufactured for Red Cross include Factor VIII Concentrate used by persons with hemophilia, albumin used to restore plasma volume in treatment of shock and burns, and immune globulins used to treat immune disorders. Red Cross plasma derivatives account for approximately 15 - 20% of the nation's supply and are produced solely from voluntary, non-remunerated donations.

Red Cross Initiatives to Reduce Recovered Plasma Pool Size

Plasma derivatives are concentrates of specific plasma proteins prepared from large pools of plasma by fractionation. Viruses are inactivated by processes such as heat and solvent-detergent treatment. I have been asked to comment on the role of plasma pool size in relation to plasma derivative safety, and to outline new safety initiatives which will ensure that Red Cross plasma products will continue to be manufactured by state-of-the-art methods.

Before doing so, it is necessary to distinguish between recovered and source plasma. Red Cross plasma derivatives are made from voluntary whole blood donations. Plasma obtained when whole blood is divided into components is called recovered plasma. In contrast, plasma derivatives made by commercial companies are manufactured principally from plasma obtained by a procedure called plasmapheresis. Plasma obtained by plasmapheresis is called source plasma.

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The Red Cross has taken several steps to reduce the number of donations in pools of recovered plasma. In early 1996 we directed Baxter to initiate and validate processes to insure that Red Cross AHF-M and IVIg (Polygam S/D) are derived from pools containing approximately 16,000 liters, or between 54,000 and 60,000 donations. Since mid-1996, the vast majority of Red Cross AHF-M and IVIg have been derived from pools containing fewer than 60,000 donations. Importantly, this process insures that the albumin used to stabilize AHF-M and IVIg is also derived from the same pool -- in other words, material from differing pools is not mixed together. Efforts will continue over the next year to reduce pool size to similar levels for the production of albumin intended for transfusion.

In addition, we are incrementally increasing the volume of recovered plasma donations through improved collection and separation techniques. Through these efforts the average volume of recovered plasma per unit of whole blood has increased from less than 250 ml to 283 ml and we expect further improvements to follow. We also intend to increase the amount of volunteer plasma obtained by plasmapheresis to further decrease the number of donors in Red Cross plasma pools.

These efforts to limit the number of donors in plasma pools will continue. Our commitment to safety is demonstrated by our record: over one billion international units of American Red Cross antihemophilic factor have been infused since the latest generation of AHF-M was introduced in 1988 with no reported cases of viral transmission.

Other Red Cross Efforts to Address Plasma Derivative Safety

Pool size is only one of the elements to consider in improving the safety of plasma derivatives. The Red Cross is actively exploring new methods to inactivate or remove potentially transmissible agents from blood and plasma such as gamma irradiation, iodine treatment, and the use of high efficiency filters. These techniques can be effective against both known and newly emergent threats to plasma derivative safety. Dr. William Drohan

of the Red Cross Holland Laboratory recently reviewed these and other technologies at a meeting of the FDA Blood Products Advisory Committee.

Within the next year, the Red Cross will also implement a highly sensitive testing technology called polymerase chain reaction, or PCR, to detect early evidence of infectious virus in plasma to be processed into derivatives. Preliminary studies suggest that PCR testing may prevent the transfusion of several hundred blood components each year that may be infectious for hepatitis C.

Product Recall Efficiency

The Subcommittee is concerned that large pool sizes impact the effectiveness of product recalls. At the November 19, 1996 FDA Informational Meeting on Notification of Plasma Withdrawals and Recalls, and at the March 1997 FDA Blood Products Advisory Committee Meeting, the Red Cross expressed its support of a system that would provide early, accurate, and complete patient notification of product recalls and withdrawals so patients can make informed decisions about their treatment.

We are acutely aware of the inadequacies in the information network that links manufacturer's product lot numbers with the patient who uses the product. For this reason we support federal regulation to mandate the permanent recording of product lot numbers by intermediate distributors, thus providing greater assurance of complete patient notification. In the absence of such a requirement, the American Red Cross has promptly notified hemophilia treatment centers and hemophilia treaters as well as the National Hemophilia Foundation of any product withdrawal or recall.

At its June 5, 1997 hearing, the Subcommittee explored the relationship between error and accident reporting (EAR) and product recalls. The timeline for initiation of a recall is distinctly different from the reporting of errors and accidents to FDA. If the Red Cross becomes aware of a problem that necessitates withdrawal or recall of a blood component or plasma derivative product, we immediately quarantine the product and do not allow its distribution. The FDA is notified of a recall as soon as we are aware of the need to retrieve product. If it has already been distributed, we immediately notify the intermediate distributor of the lots involved and ask that they in turn notify the prescribing physician. Gaining control of the product as soon as possible is the most important action.

Filing Error and Accident Reports is a separate action, decoupled from the product recovery action and recall notification with FDA. Red Cross has established a Recall Task Force to review recalls and market withdrawals, with the objective of reducing them to the greatest extent possible.

Regulatory Issues

The Red Cross blood and plasma programs are regulated by the Food and Drug Administration. We are inspected by the FDA Office of Regulatory Affairs and by several other governmental and professional organizations. Since 1993 the Red Cross has been operating under a consent decree agreed to by the Red Cross and the FDA that is designed to improve our operations in several key areas. We have essentially completed all requirements of the consent decree. For example, we have consolidated our 50 testing laboratories into nine new standardized state-of-the-art facilities that test all blood donated to the Red Cross. We have also developed a powerful quality assurance program that is the model for the industry. The FDA has been very tough but fair throughout this process. The Red Cross is now a stronger, better managed, more efficient organization because of these efforts.

Creutzfeldt-Jakob Disease (CJD)

I have been asked to address the evidence regarding the potential for transmission of CJD through the blood supply, and to review Red Cross research in this area. The Red Cross takes all potential threats to blood and plasma safety very seriously, and we have moved aggressively to expand the body of scientific information related to CJD. We have several research studies underway at our Holland Laboratory and in collaboration with Dr. Paul Brown at NIH and Dr. Robert Rohwer at the Veterans Administration. The Red Cross has committed over $1 million in research studying possible links between CJD and transfusion, probably more than any other private organization.

We have also taken steps to reduce the likelihood that plasma from a donor subsequently diagnosed with CJD is included in pools for fractionation. CJD is a disease of older people, with a mean age of incidence of 67. The Red Cross only uses plasma from donors 59 years old or younger for fractionation, thus eliminating the age group at greatest risk from plasma pools. Plasma from older donors continues to be used beneficially as single donor products.

The Red Cross is also conducting a CJD "lookback" study under the direction of Marion Sullivan at the Red Cross Holland Laboratory. She has studied 179 recipients of blood transfusions from donors subsequently diagnosed with CJD. These recipients have been followed for up to 25 years following transfusion. None of the recipients has died of CJD or shown any sign of the illness.

These data are encouraging. Until there is further convincing evidence of non-transmissibility, however, the Red Cross will continue to quickly withdraw plasma derivatives following receipt of post-donation information from a donor or a donor's family about a risk of CJD.

Conclusion

The American Red Cross is committed to providing an adequate supply of blood components and plasma derivatives that meet the highest standards of safety. Red Cross plasma derivatives have proven to be both safe and effective. We have taken steps to insure this safety by reducing the number of volunteer recovered plasma donations in pools for fractionation, and by eliminating plasma from donors in age brackets most likely to affected by CJD. These steps are part of a larger program to improve safety by an aggressive quality assurance program, focused research programs and improved donor screening and testing. The Red Cross takes the issue of blood safety very seriously. We are proud of our record and of our tradition of serving the American people.

Thank you.

Mr. SHAYS. Mr. Reilly.

Mr. REILLY. Thank you. My name is Robert Reilly. I'm the executive director of the International Plasma Products Industry Association [IPPIA]. Thank you for the opportunity to testify today. We applaud you and the subcommittee for your work and oversight in the complex area of maintaining the safety of our Nation's blood supply. We view ourselves as partners in the effort to give the highest possible assurance to people who depend upon plasma-based therapies that safety is of paramount importance to us.

Chairman Shays, we heard you at the November 1995 hearing, on the subject of protecting the Nation's blood supply when you cited the need for leadership. As providers for plasma-based therapies, we are, and must continue to be, leaders in that commitment to safety. It is a responsibility that we take very seriously.

I would like to begin by briefly describing the four main areas of focus in my testimony: First, the role of pool size as a component in the overall layers of safety. Second, the series of public policy issues that examine the fragile balance among safety, availability, and efficacy. Third, an industry initiative reducing pool size. Finally, we want to discuss our continuing commitment to make plasma-based therapies safer still.

During testimony before a 1993 hearing of this subcommittee, FDA described five traditional layers of safety. Our industry can, and does, go beyond those five basic layers and employs additional layers. Each of these layers is a defense against both known and unknown agents. Together they form a protective safety barrier that is far stronger than each of the component parts. Yet all of the parts must be strong in order to provide the best assurance of safety.

Let me summarize our industry programs and how they complement each of the layers of safety. Since its inception in 1991, the Quality Plasma Program [QPP] has required that all applicant donors undergo additional AIDS/high risk education and screening. The industry has introduced a series of four voluntary standards, the first of which requires that no unit of plasma be accepted for further processing unless the donor has successfully passed at least two health assessments. Plasma from one-time donors will not be accepted under this new standard. All companies and facilities maintain registries of donors who have been previously deferred for a variety of reasons.

Beyond this, the American Blood Resources Association developed and received an FDA 510(k) authorization to operate the National Donor Deferral Registry.

In addition to the specific FDA required tests, the industry is in the process of adopting Genome Amplification Technology, commonly known as PCR. A substantial improvement in this layer is the addition of a 60-day hold of plasma inventory, announced as one of the voluntary standards introduced by the IPPIA members. This voluntary standard will be fully implemented by year-end.

Our quality assurance procedures provide a method to constantly monitor and further improve the margin of safety of plasma-based therapies. For example, QPP has set a standard that measures each collection center on an industry-wide basis.

Each of our member companies commits a significant investment of human and financial resources devoted to this area. In addition to the companies—in addition to the viral inactivation/removal procedures, the companies have created a consortium for plasma science which is providing additional funding for research focused on source plasma pathogen inactivation technologies.

With respect to notification/recall, the industry is developing a well-publicized industry Web page with detailed information on plasma-based therapies. In addition, IPPIA is developing a formal network of user groups designed to directly contact consumers of plasma-based therapies. Together these layers form a web of protection against both the known and the unknown risks we face now and in the future.

In addition, we must examine the balance between safety, efficacy, and availability. We must, however, balance those things carefully.

In regard to product availability, we have to consider the effect limiting pool size may have on access to plasma-based therapies. The accompanying chart illustrates the effect on the supply of plasma-based therapies of an immediate application of FDA limits on donor exposures to 15,000. The chart shows the actual consumption for four major therapies for the calendar year 1996. Superimposed on each bar is the percent of each product industry would be able to produce under this limit. As you can see, the FDA's suggestion would seriously compromise industry's ability to provide an adequate supply of these life-saving therapies. The efficacy of the therapies, how well they perform, is important——

Mr. SHAYS. I'm going to interrupt you a second. If I forget to ask that question, I'll need that explained——

Mr. REILLY. Yes, sir.

Mr. SHAYS [continuing]. As to why that's the case.

Mr. REILLY. The efficacy of the therapies, how well they perform, is an important element in our consideration of the impact of pool size. That is why the effect of any changes in pool size on the efficacy and quality characteristics of these therapies must be aggressively monitored and studied to ensure that plasma-based therapies remain effective in treating the patients who rely upon them.

Industry has taken an initiative to reduce the pool size. The industry recognizes the role of donor exposure and pool size in balancing the needs of product safety, availability, and efficacy. We have worked diligently to develop an industry effort to limit the number of donors to which patients are exposed. Our IPPIA proposal recognizes that, from data that we have collected, that donor exposures of some therapies can exceed the 100,000 level. We're now confident that we can improve that situation. The IPPIA companies have committed to developing and implementing enhanced manufacturing practices for the major therapies. This will have the immediate impact of at least a 40 percent reduction in such levels of donor exposure.

We believe that we can achieve this without creating product shortages, without major plant reconstruction or renovation, and without a lengthy FDA process that would otherwise delay implementation of these—of this initiative. Where, long-term, our members are committed to work with FDA product by product, company

by company to further reduce the risks associated by donor exposure. That may require amendments to product licenses, plant reconstruction, or plant renovations. The industry will report on the continuing improvements being made in this area.

What we are pursuing beyond our pool size initiative is a comprehensive plan that builds upon the seven layers of safety that I have just mentioned. That is our goal, our challenge and commitment. We will in the future verify the successes of our efforts and—through accurate reporting measurements.

People who depend upon plasma-based therapies for their health and their very lives can be reassured the industry is working diligently that those therapies are safe, available, and effective.

We commend you, Mr. Chairman, for your leadership in provoking dialog on this important issue which has resulted in improvements in the Nation's blood supply. Thank you.

Mr. SHAYS. Thank you.

[The prepared statement of Mr. Reilly follows:]

Statement of Robert W. Reilly, Executive Director
International Plasma Products Industry Association
July 31, 1997

Mr. Chairman:

My name is Robert W. Reilly. I am Executive Director of the *International Plasma Products Industry Association (IPPIA)*, the international trade association representing the commercial producers of plasma-based therapies. Our members include Alpha, Baxter, Bayer, and Centeon and represent nearly 80% of all the plasma-based therapies producers in the United States.

Thank you very much for giving me the opportunity to testify before this subcommittee today.

The subject of this hearing involves issues that are literally of life and death importance to the patients our industry serves. We welcome this chance to discuss the plasma-based therapies produced by our member companies and industry's efforts to ensure quality and safety for the patients who rely upon those therapies.

We applaud you and the subcommittee for your work and oversight in the complex area of maintaining the safety of our nation's blood supply. We view ourselves as partners in the effort to give the highest possible assurance to the people who depend upon plasma-based therapies that safety is of paramount importance to us.

Congressman Shays, we heard you at the November 1995 hearing on the subject of protecting the nation's' blood supply when you cited the need for leadership. As providers of plasma-based therapies we are, and must continue to be, leaders in that commitment to safety. It is a responsibility that we take very seriously.

The purpose of my testimony today is to outline how the industry has responded to that call by building upon its strong resolve to provide safe and effective therapies to the patients who depend upon them.

I would like to begin by briefly describing the four main areas of focus we believe should be included in a comprehensive discussion and review of pool size as it relates to the manufacture of plasma-based therapies.

First we will discuss the role of pool size as a component in the overall layers of safety used in the manufacture of plasma-based therapies.

Second is a series of public policy issues that must be considered in conjunction with proposals to limit pool size -- issues that cause us to examine the fragile balance between *safety*, *availability* and *efficacy*.

Third is an industry initiative addressing the pool size issue, which I would like to outline for the subcommittee. This initiative underscores our commitment to work with you and the FDA to reduce risks associated with plasma pool size.

Finally, we want to discuss more broadly the industry's strategic objectives and voluntary initiatives that demonstrate the industry's continuous commitment to making plasma-based therapies safer still.

I. SEVEN LAYERS OF SAFETY

In a 1993 hearing of this subcommittee, the Food and Drug Administration (FDA) described five traditional layers of safety for blood products. Certainly we agree with the validity of those layers, but our industry can and does go beyond the five basic layers of safety. The uniqueness of fractionation allows for these additional layers of safety that we believe are fundamental to achieving the level of safety our patients expect and need.

[Reference Chart A]

- **Donor Screening**
- **Donor Deferral**
- **Donor Testing**

- **Inventory Hold**
- **Quality Assurance & Good Manufacturing**
- **Viral Inactivation/Removal**
- **Recall/Notification**

Each of these is a layer of defense against both known and unknown agents. They form a protective safety barrier that is stronger than each of the component parts. Yet, all of these parts must be strong in order to provide the best assurance of safety. Therefore, the industry has actively and methodically undertaken a series of voluntary initiatives to address these opportunities for defense.

In 1991, industry initiated a program known as the Quality Plasma Program (QPP) certification under the umbrella of the American Blood Resources Association (ABRA). QPP has been adopted by nearly 100% of the FDA licensed plasma collection facilities and has been updated and improved upon several times. More recently the safety of plasma-based therapies has been enhanced by a series of four voluntary industry standards adopted by all of the members of the IPPIA.

Each of the expanded layers play an important role in improving the safety of plasma-based therapies and can be summarized as follows:

[Reference Chart B]

1. Donor Screening

Since its inception in 1991, QPP has required that all applicant donors undergo additional AIDS/high risk education and screening. This includes a variety of measures ranging from additional educational materials to an assessment of the applicant donor's comprehension of these risk factors and the need to self-defer from donation.

Additionally, QPP requires that facilities add screening criteria intended to encourage repeat donations by low risk populations in the facility's local community. Repeat donations by a qualified donor population enhances confidence in the safety of these donors.

To further ensure and enhance this point, the first industry voluntary standard requires that no units of plasma be accepted for further processing unless the donor has successfully passed at least two health history interviews. This standard, on top of the previous educational and donor interview procedures, provides further improvement to the quality of plasma from donors. Further, it provides the ability to retrospectively assess the acceptability of the initial donation with subsequent interview results. Finally, this completely eliminates the use of plasma from one-time donors, who are widely recognized as being at higher risk of infectivity.

2. Donor Deferral

All companies and facilities maintain registries of donors who have been previously deferred for a variety of reasons. Beyond this, ABRA has designed, developed, and received an FDA 510(k) review and authorization to operate the National Donor Deferral Registry (NDDR). The NDDR incorporates modern computer technology to allow all commercial plasma collection facilities to share donor deferral information on a confidential, inter-company, nationwide basis for the purpose of checking applicant donors in advance of their donation.

The NDDR provides two substantial benefits: first, it excludes from further donations any individual who was previously deferred because of positive viral marker tests; and second, the NDDR limits unnecessary exposure of collection center and laboratory personnel to known positive units of plasma or test samples.

3. Donor Testing

In addition to the specific FDA required tests, the industry is in the process of adopting the Genome Amplification Technology, commonly known as Polymerase Chain Reaction (PCR). Studies have shown this technology can further reduce the "window period." Each of the manufacturers is working closely with FDA and other affected parties to either approve or implement PCR technology as rapidly as possible.

The voluntary standard requiring a second donor screening interview also requires applicant donors to be subjected to two rounds of testing by both existing FDA required test methodologies, and in time, PCR testing.

4. Inventory Hold

FDA has always required facilities to hold all units of plasma until viral marker test results are available. The addition of PCR testing will further strengthen the efficacy of that holding period.

Yet, another substantial improvement to this layer is the addition of a 60 day hold of plasma inventory announced as a voluntary standard by the IPPIA members. This voluntary standard will be implemented by year end [Reference Chart C]. This standard provides a guaranteed look-back period so that whenever a qualified donor seroconverts on a subsequent donation, or new health history information is discovered, both the present and previous donations are removed from the manufacturing process.

5. Quality Assurance & Good Manufacturing

Our quality assurance procedures provide a method to constantly monitor and improve the safety of plasma-based therapies. For example, QPP sets a standard to measure each collection center on an industry-wide level. That standard has been tightened twice since its inception in 1991, and is currently being completely redesigned and enhanced once again. This enhanced standard will focus on a measurement of the industry-wide viral marker rate.

6. Viral Inactivation/Removal

IPPIA members are committed to seek out and use the most advanced technology available in their viral inactivation and removal processes. Each of our member companies commits a significant investment of human and financial resources to this area. In addition to this individual research and development, the companies have created the Consortium for Plasma Science, which is providing additional funding for research focused on source plasma pathogen inactivation technologies.

7. Recall/Notification

In the area of patient notification, the industry is developing better methods to efficiently and effectively notify patients with important information about our plasma-based therapies. Specifically, industry is developing a well-publicized industry web page with detailed information on plasma-based therapies. The

page will have hyperlinks to other pages with important industry information. In addition, IPPIA is developing a formal network of user groups designed to directly contact consumers of our therapies. We are working together with all interested parties and have received initial favorable responses to our initiative.

As you can see through our series of industry initiatives (QPP, NDDR, voluntary standards, etc.) industry has proactively developed a comprehensive program aimed at each of the seven layers of safety. This comprehensive system provides a web of protection against the known and unknown risks that we face now and in the future.

II. BALANCING SAFETY, AVAILABILITY, & EFFICACY

Just as these additional layers of protection are integral parts of an overall strategy to maintain and improve the safety of plasma-based therapies, each of them also is important to a comprehensive discussion of limiting pool size. Together these components of safety provide a key element of the public policy considerations that are appropriate to think about in the context of limiting pool size.

One way to look at those considerations is as a three-legged platform, with the legs being safety benefits, product availability, and the efficacy of the product. In order to provide a balanced and stable policy platform, the legs must be of compatible length and strength. We must balance safety with the importance of availability and efficacy. Without it, the platform will not perform to its best

potential, and the needs of the patient community, which must be our first concern, will not be met.

The issue of safety is of immense concern to the industry, and our efforts emphasize the importance of that leg of the policy platform in achieving balance with the concerns of product availability and efficacy. Regrettably, there is no simple formula for finding the complex balance point that measures the increments of safety gained in availability and efficacy of plasma-based therapies.

In regard to product availability, we have to consider the effect of limiting pool size on the ability of the industry to provide sufficient quantities of plasma-based therapies. The accompanying chart [Reference Chart D] illustrates the effect on the supply of plasma-based therapies of an immediate FDA suggestion to limit donor exposure to 15,000. Under present conditions, some companies simply would not be able to continue to manufacture these therapies. The chart shows the actual consumption for the four major therapies for the calendar year 1996. Superimposed on each bar is the percent of each product industry would be able to produce under this limit. As you can see, the FDA suggestion would seriously compromise industry's ability to provide an adequate supply of these life-saving therapies.

The FDA suggestion would require manufacturers to process plasma in smaller starting pools in order to decrease donor exposures to this level. However, certain manufacturing equipment such as freeze dryers, require the same amount of time in the manufacturing process regardless of the size of the starting pool. This equipment limits the number of smaller pools that can be processed by the facility, which, therefore, limits the overall output.

Because of increasing demand for plasma-based therapies, these plants are already running at full or near-full capacity. In order for industry to be able to manufacture even the equivalent of the 1996 supply of these therapies under this limit, drastic changes to the manufacturing process would be required. For some equipment, such as ultrafiltration devices, the space for each piece of equipment to run a smaller volume does not decrease. In fact, our investigations have shown that the physical space, machinery, electricity and water required for the production of these therapies increases at near geometric rates as batch size is reduced to these levels.

Existing equipment that might be usable at reduced capacity would also require re-validation and re-approval from the FDA for use with these smaller volumes. Significant plant reconstruction to provide the needed space and a lengthy FDA approval process would require at least three to five years and probably longer. Furthermore, the number of people to support each of these activities also increases, causing a greater opportunity for Good Manufacturing Practice (GMP) errors.

The efficacy of the therapies -- how well they perform -- is an important element in our consideration of the impact of pool size. We will have achieved an unsatisfactory "balance" if we provide a safe product in sufficient quantity that does not do the job for which it is intended at the level of benefit our patient groups need and expect from us. That is why the effect of any changes in pool size on the efficacy and quality characteristics of these therapies must be aggressively monitored with a constant eye toward ensuring that plasma-based therapies are effective in treating the patients who depend upon them.

III. INDUSTRY INITIATIVE TO REDUCE DONOR EXPOSURE

The industry recognizes the role of donor exposure and pool size in balancing the needs of product availability, safety and efficacy. We have worked diligently to develop an intensive industry effort to limit the number of donors to which patients are exposed when using plasma-based therapies. Those efforts have evolved and intensified over the past year.

Part of that evolution was the consideration of the basic unit of measure by which we define pool size. We believe that the most accurate unit of measure is the total number of donors to which a patient is theoretically exposed when using a plasma-based therapy. That measurement must also take into account the use of additives or stabilizers in the manufacturing process that create exposure to additional donors. As a result, all of the numbers we cite are measured by total donor exposure.

We recently provided data to the Food and Drug Administration for all major product lines, including Factor VIII, Factor IX, Albumin, and IVIG, which show the total donor exposure for these therapies. The numbers vary significantly from manufacturer to manufacturer and from product line to product line. That is a result of the unique characteristics of each plasma-based therapy and the unique manufacturing processes used by each provider of the particular therapy.

While we remain vigilant to maintain the delicate balance among safety, product availability and efficacy of these therapies, the industry has performed sufficient research for us to commit ourselves to reducing donor exposure significantly. The data we have shows, for example, that donor exposure to some therapies can exceed 100,000. We are now confident that situation can be improved. Our IPPIA member companies are committed to developing and implementing enhanced manufacturing practices for the major therapies (Albumin, Immunoglobulins, Factor VIII, and Factor IX). This will have the immediate impact of at least a forty percent reduction in such levels of donor exposure.

We believe we can achieve this without creating product shortages, without major plant reconstruction or renovation, and without a lengthy FDA approval process that could delay implementation of this initiative. This could be accomplished while maintaining compliance with Good Manufacturing Practices.

More long term, our members are committed to working with the FDA, product by product, company by company, to further reduce the risks associated with donor exposure. Some of those additional initiatives may require amendments to product licenses, plant construction, or plant renovation.

We cannot guarantee overnight success in these additional endeavors, but we expect to see progress within a year. The industry will welcome an opportunity to sit down with the FDA and report to the agency the continued improvements we are making in reducing the risks associated with donor exposure.

IV. INDUSTRY COMMITMENT TO SAFETY

What we are pursuing -- and we believe it is completely consistent with your own strong personal interest in this issue, Mr. Chairman -- is a comprehensive plan that builds upon the seven layers of safety I mentioned at the beginning of my testimony. That is our goal, our challenge, and our commitment -- and we will verify the success of our efforts through accurate measurements.

Mr. Chairman, we did hear you at the prior hearing when you called for the need for leadership and challenged the industry to deal with the issue of plasma pool size. We have worked since then to develop an industry initiative and are now on the brink of its implementation.

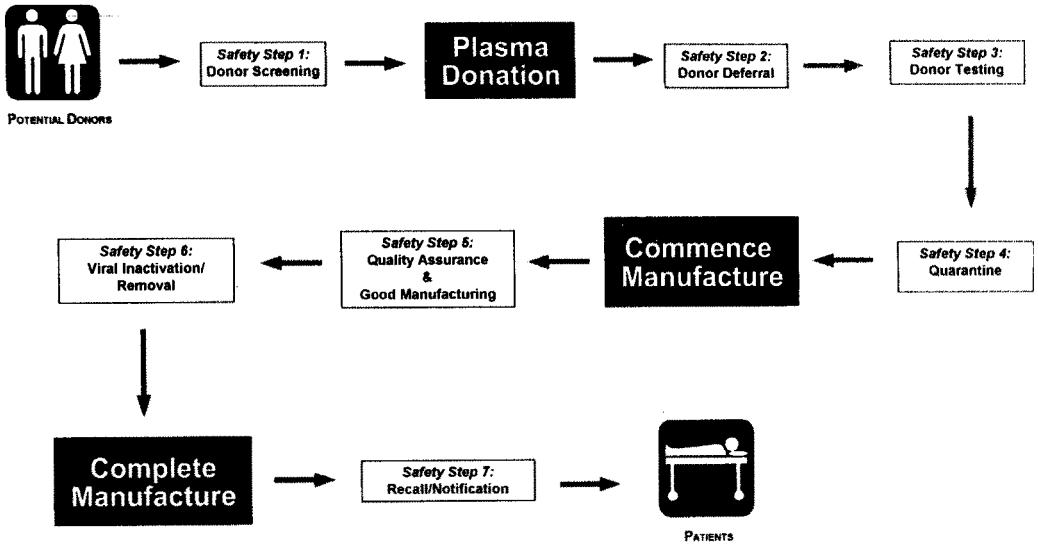
The industry is involved in a series of multi-faceted voluntary initiatives to which we now pledge to expand our comprehensive plan to further increase the margin of safety for plasma-based therapies. The seven layers of safety are a foundation upon which we are building in our on-going commitment to making plasma-based therapies safer still. The industry is dedicated to continuous improvement so that the people who depend upon plasma-based therapies for their health and their very lives will know that those therapies are safe, available, and effective.

We commend you, Mr. Chairman, for your leadership in provoking dialogue on this important issue which has resulted in improvements in our nation's blood supply.

Thank you and I will be happy to answer your questions.

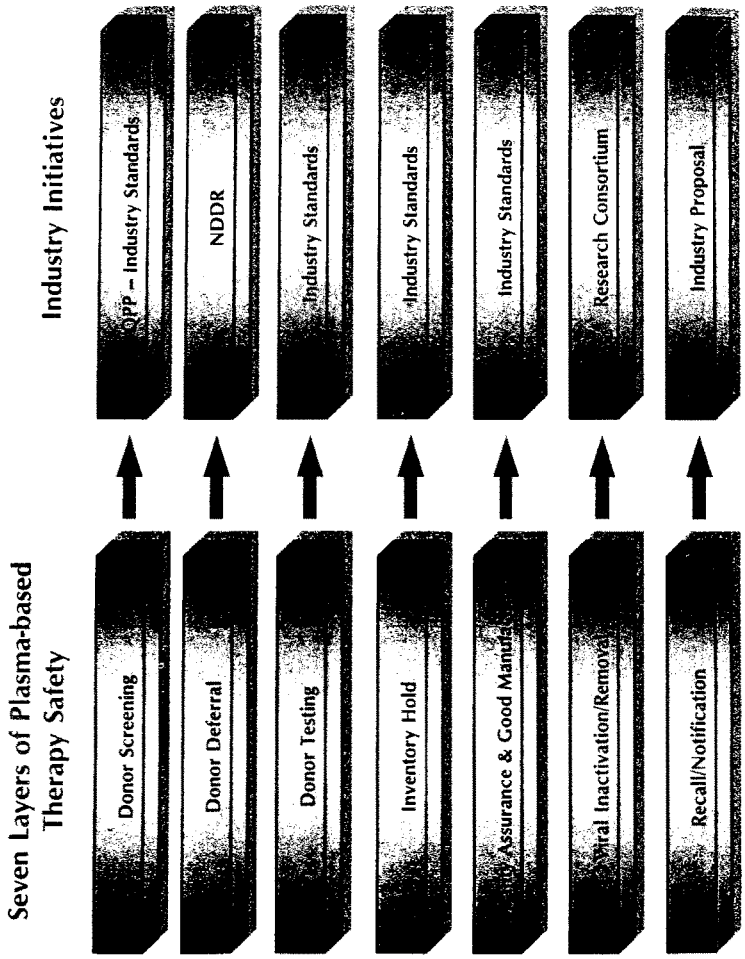
Referenced Charts

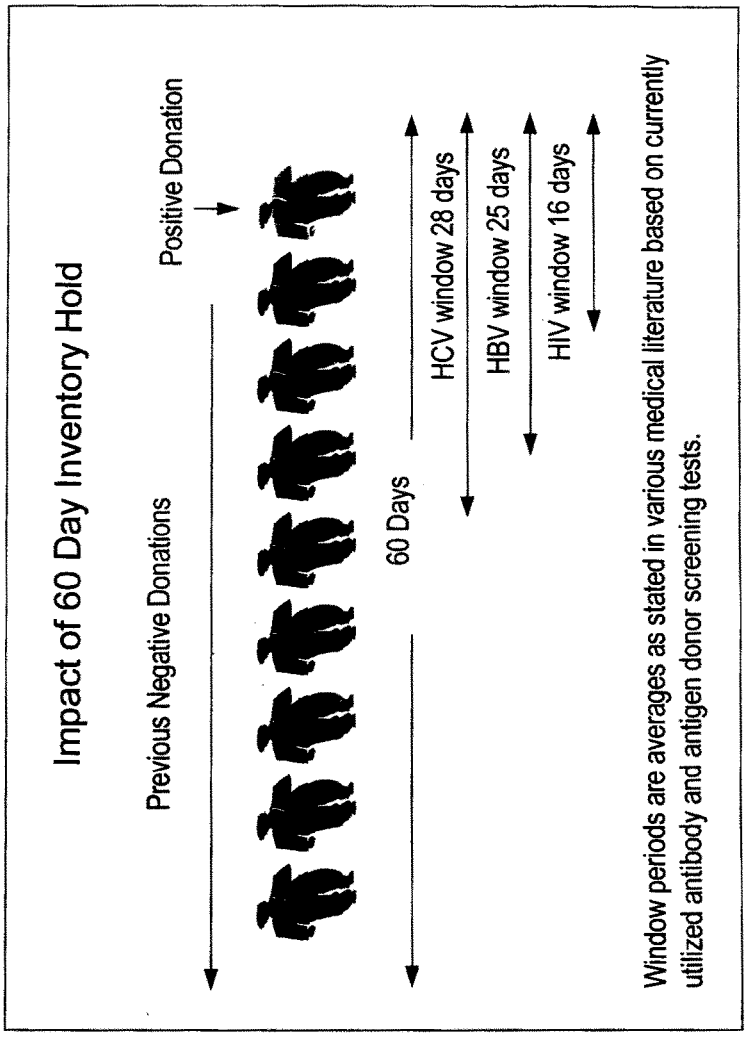
Fractionation Safety



Prepared by Georgetown Economic Services

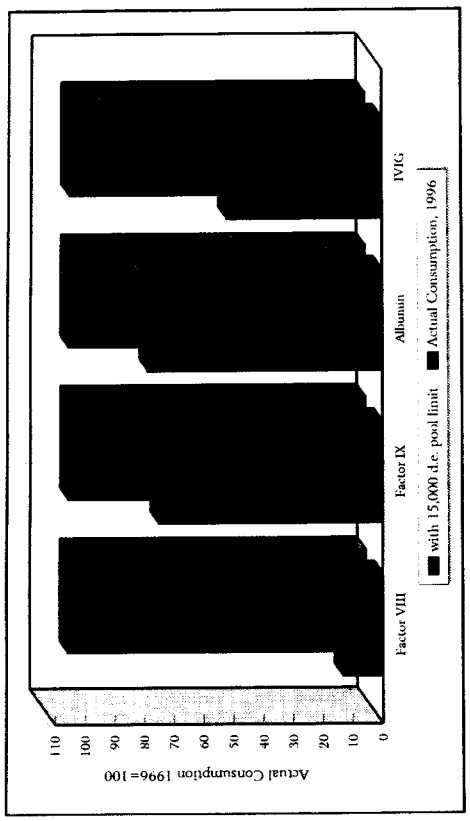
B


Seven Layers of Plasma-based Therapy Safety
Industry Initiatives
Donor Screening
Donor Deferral
Donor Testing
Inventory Hold
Assurance & Good Manuf.
Viral Inactivation/Removal
Recall/Notification
OPP – Industry Standards
NDDR
Industry Standards
Industry Standards
Industry Standards
Research Consortium
Industry Proposal


Impact of 60 Day Inventory Hold
Previous Negative Donations
Positive Donation
60 Days
HCV window 28 days
HBV window 25 days
HIV window 16 days
Window periods are averages as stated in various medical literature based on currently utilized antibody and antigen donor screening tests.

D

Impact of 15,000 Donor . .osure Pool Limit
on U.S. Based Plasma Products Availability
1996



Source: Marketing Research Bureau and Fractionator data.

Prepared by Georgetown Economic Services

Mr. SHAYS. Just since I had raised it, I'm not aware that anyone has suggested limiting the pool size to 15,000. I mean, this will be interesting dialog. But it's not something I'm aware of that is being——

Mr. REILLY. The 15,000 number is a result of a recommendation from FDA discussed at an earlier blood products advisory committee meeting.

Mr. SHAYS. OK. That they would limit it to 15,000?

Mr. REILLY. Yes, sir.

Mr. SHAYS. OK. Do I pronounce your name Fournel?

Mr. FOURNEL. Fournel.

Mr. SHAYS. Thank you. I think I didn't pronounce properly the first time. Mr. Fournel, thank you.

Mr. FOURNEL. Mr. Chairman, and members of the subcommittee, I would like to thank you on behalf of Bayer Corp., for inviting us to return to these hearings. We commend you for the leadership you have shown in your continuing efforts to safeguard our Nation's blood supply. In 1995, Bayer pledged to work with you and the other members of this subcommittee. Today, we reaffirm this commitment.

For the past 20 years, I have conducted research and development of plasma products that are used to manage serious illnesses that in many cases respond only to the proteins found in human plasma.

Mr. Chairman, at Bayer, the development and application of new technologies to improve safety for patients is at the very core of our business. Since my last appearance before this committee, Bayer has continued to move forward. I would like to describe four examples of these efforts, which, together, have the potential to enhance the margin of safety for the patients who use our products.

First, Bayer is developing genome amplification technology for use in detecting viral genomes in individual plasma donations. We are filing an IND to use a preliminary chain reaction, or PCR, test for the detection of hepatitis C virus in plasma.

Our initial research shows that PCR will help to reduce the window period during which infection may be present, but detectable levels of viral antibodies or antigens have yet to appear. We expect that full use of PCR testing will reduce potential viral loads, which in combination with our validated clearance studies will provide an additional safety margin for our products.

Second, because first-time donors have a higher probability of viral infection than repeat donors, Bayer has recently placed special restrictions on plasma collected from them. We destroy all plasma from such donors if they do not return to make a second donation. This means that we will accept plasma only from qualified repeat donors.

Third, Bayer has established an inventory hold program for all plasma units. We will store plasma for a minimum of 60 days before use. Should a donor on a repeat visit center to a plasma center fail our testing or screening procedures, we will retrieve and destroy all previously qualified units of plasma from that donor in our inventory. This program will reduce the possibility of a window case of hepatitis or HIV from contaminated plasma supplies.

Fourth, the subcommittee has expressed concern about potential emerging infectious agents, particularly Creutzfeldt-Jakob disease. Bayer continues to invest its major scientific efforts to address these challenges. Our CJD work specifically centers on development of assays capable of detecting the potential, if any, for transmission of CJD in plasma products, process clearance studies to identify process methods with the greatest ability to remove pathogens that may be present in plasma, and potential transmission risk studies to define the clearance requirements, if any, for processing steps.

The subcommittee has also asked Bayer to provide views on regulating the size of plasma pools, what I will call donor exposure in a final container product. Bayer agrees with the preceding testimony by the industry association. For most products, Bayer currently outperforms this commitment and will continue to strive to reduce donor exposure in all of our products.

As you know, this is a complex issue which I will illustrate with an example of a product, Prolastin, for people with a genetic deficiency associated with emphysema. Successful therapy requires weekly injections of Prolastin. Thus the health of these patients depends not only on this product's safety, but also on its availability.

To assure that Prolastin is available to as many patients as possible, we now use about 60,000 donor exposures per final container product. There are several reasons for this. First, a unit of human plasma contains only a small fraction of the protein missing in those who use Prolastin. If everyone in this room donated plasma today, we could provide only a 1-month supply of this treatment for one patient.

Second, scarce resources require efficient processing. Bayer relies on the economies of large-scale pool fractionation to obtain as much product as possible. The FDA has suggested a 15,000 donor exposure limit. Given the constraints of the technology and our current license for processing Prolastin, we estimate immediate implementation of this limit would cut our product availability in half. Further, making this reduction without breaking the product lifeline would require custom-built equipment, validation trials, and FDA approval of all changes in manufacturing processes. We estimate it would require several years to accomplish these changes. Meanwhile, we expect that our current efforts to improve yields from plasma, if successful, will achieve similar reductions in donor exposure without limiting supply.

To fully demonstrate the science and vigilance behind our plasma products, I would like to invite the subcommittee members and staff to tour Bayer's facilities and fractionation plant near Raleigh, NC.

We believe, Mr. Chairman, that a rational approach toward improving product safety, which integrates both effective material management to reduce donor exposure and measures like those I've outlined today, affords the best opportunity to achieve our common goal of reducing safety risk to the patients who depend upon these life-saving therapies.

Thank you.

Mr. SHAYS. Thank you very much.

[The prepared statement of Mr. Fournel follows:]

Full Statement of Michael Fournel
Bayer Corporation to the
Subcommittee on Human Resources
July 31, 1997

INTRODUCTION

Mr. Chairman and members of the Subcommittee, I would like to thank you on behalf of Bayer Corporation for inviting us to return to these hearings and address you again. We commend you for the leadership you have shown in your continuing efforts to safeguard our nation's blood supply and the resulting plasma products.

We share your mission and recognize that this process works in the public interest. All of us are potential beneficiaries of the life saving therapies that are the subject of this congressional panel's inquiry. In 1995, Bayer made a commitment to work with you and the other members of this Subcommittee. Today, we reaffirm this commitment.

OVERVIEW OF BAYER

My name is Michael Fournel. I am Vice President of Research and Technology for Biological Products in the Pharmaceutical Division of Bayer Corporation, where I have conducted research and development efforts on plasma products for more than 20 years. During this time, I have been responsible for the pre-clinical development of a series of Bayer products derived from plasma fractionation and biotechnology.

I will begin by describing briefly my company and its role in this field. Bayer is a research-based company with major businesses in life science, chemical and imaging technologies. Plasma and biotechnology derived products comprise Bayer's biologicals business. These include Gamimmune N® for treatment of immunocompromised patients, Prolastin® for treatment of alpha$_1$ deficiency or genetic emphysema, and Kogenate® for the treatment of hemophilia A. This business is part of Bayer Pharmaceuticals, a division of Bayer Corporation. Bayer Corporation, with headquarters in Pittsburgh, is a wholly owned subsidiary of Bayer AG, based in Leverkusen, Germany.

Ever since Bayer developed Aspirin a century ago, our commitment to research has been a part of our identity as a pharmaceutical company. We also are guided in our work by our focus on products that contribute to the well-being of the peoples in our society: curing and preventing disease and enhancing the quality of life. Equally important, however, has been our ongoing commitment to ensuring the quality of our products: for our biologicals business, this includes developing and applying new technologies and methods which are intended to continually improve the safety of plasma products.

FOUR AREAS OF PROGRESS SINCE 1995 HEARINGS

Since our appearance before this subcommittee in 1995 Bayer has undertaken many initiatives intended to meet our common goals of improved donor and patient safety, some of which were begun before that appearance. Today, I would like to outline four specific examples of new initiatives we have implemented since my last appearance and detail the impact of these incremental improvements on the overall product quality and safety of Bayer's biological and biotechnology products.

1. First Time ("Applicant") Donors

It has been recognized that first time or applicant donors have a higher probability of presenting with a viral infection than repeat donors; for example, individuals may present for plasmapheresis solely to obtain a free test result to ascertain their health status. In 1995, this Subcommittee heard testimony that the majority of plasma donations that test positive for HIV or Hepatitis B or C (using currently approved surrogate tests) come from first time donors. In recognition of this phenomenon, Bayer recently placed special restrictions on plasma collected from first time donors (individuals who wish to become donors and have not applied during the previous six months). Even if the first time donor meets all health standards and the plasma collected passes all tests, we now require a repeat donation from the individual and only upon successful passage of repeat health check and plasma testing will this individual's plasma be used for the manufacture of our products. As a precaution, we destroy all plasma from first time donors who do not return to make a second donation within the six month time-frame.

While this program will result in a significant loss of plasma (which was previously collected for use in our processes) with the associated costs, the anticipated incremental improvement in safety warrants this change for the benefit of the recipients of our products.

2. Inventory Hold

The current state of the art for identifying viral infectivity in plasma is the use of FDA approved tests which measure either a protein fragment of the specific virus (HBsAg or HIV-1 antigen) or the host's response to a viral infection (anti-HCV or anti-HIV antibody, elevated liver enzymes). While quite reliable as indicators of infection, these tests are surrogates for the detection of actual virus. In most cases, they become positive some time after an active infection occurs. Since infectivity of plasma due to viremia can occur prior to development of a positive surrogate test, a "window" of infectivity can exist in plasma collected sequentially from a donor who develops such an infection. This "window" period varies with different viruses but in general can be as long as 50 days.

In response to this reality, Bayer has instituted inventory controls which results in the holding of plasma donations for at least 60 days before release to pooling and subsequent fractionation into our products. Should a donor, during a repeat visit to a plasma center, show evidence of infection using the current FDA approved tests (or present or report other unacceptable characteristics), we will retrieve and destroy all units of plasma in our inventory from this donor. This innovative program has the potential to reduce the risk of manufacturing products from donors in a seroconversion window for Hepatitis or HIV and delivers yet another integrated layer of safeguards to our overall product quality and safety profile. While there are considerable financial costs and overall reduced product availability as a result of this initiative, we believe increasing inventory hold greatly contributes to a reliably safe and consistent plasma supply.

3. PCR Testing

While present methods for the detection of viral infection have significantly reduced the occurrence of infectivity in blood and plasma products, shortcomings of existing tests such as those mentioned above fuel the drive for new and better methods. Additionally, new detection technologies are emerging that will form the platform for the next generation of donor screening tests. Gene amplification methods such as the polymerase chain reaction (PCR) offer the promise of high specificity, sensitivity and early detection of infectivity in plasma, effectively narrowing the viremic window period. Bayer is committed to the introduction of such testing as soon as possible.

The PCR test uses gene amplification technology to detect the presence of nucleic acid from individual infectious agents. Bayer's investigations into PCR are focusing on the ability of this advanced screening technology to identify individual positive units that could potentially enter the plasma pool. We anticipate these investigations with PCR may help to reduce the "window" period during which infection may be present but detectable levels of viral antigens or antibodies have yet to appear. We hope that full use of PCR testing may reduce viral loads to less than 1000 genome copies per milliliter (that is, the detection limit of the test); in the case of HCV, combined with our validated process viral clearance capabilities, we then would predict a multi-million fold safety margin from Hepatitis C infection in products produced from HCV PCR negative plasma donors.

To expedite implementation of this testing, Bayer has made substantial resource and capital investments including the construction and staffing of a state-of-the-art testing facility in Raleigh, North Carolina. We are filing an IND for utilization of this technology to test for HCV on individual plasma samples and hope for full implementation on all of our plasma collected by year end, and implementation of testing for HIV in 1998. Bayer's ultimate goal is to continuously expand the portfolio of pathogens detected by PCR technology to include all currently tested pathogens.

4. CJD Research

The Subcommittee has expressed concern about potential emerging infectious agents, exemplified by Creutzfeldt-Jakob Disease. Unlike conventional pathogens, such as viruses, there are many unanswered questions in the science and technology for studying CJD or other transmissible spongiform encephalopathies (TSE). The agent(s) responsible for the transmission of TSE diseases have not been completely identified, nor are well-characterized reagents available to conduct assays for the protein marker of the disease, PrP^{RES}. Moreover, no *in vitro* assays are available (current animal assays require nearly a year to generate results), nor are there any early detection markers available for identification of TSE diseases in asymptomatic individuals.

In response to this status, Bayer has initiated a major scientific effort to address this challenge. This work centers on three elements:

1. **Improved Assay Development.** Our goal is to find assays capable of detecting the potential for transmission of CJD, as well as the causal agent involved in TSE diseases. Current prion assays depend on detection of pathology developed in the brains of inoculated animals. As such, each assay can take over nine months to generate results. Development of new or improved *in vitro* assays for protease-resistant prion protein (PrP^{RES}) will permit identification and quantification of this protein in samples. The goal is to find a potential surrogate for infectivity assays, as well as an early marker of TSE diseases in asymptomatic individuals. Successful assay development could represent a significant contribution to the scientific field.

2. **Process Clearance Studies.** The goal is to identify process methods with the greatest potential to remove pathogens that may be present in plasma. In these experiments, researchers intentionally contaminate in-process material in an experimental setting and subject it to various processing steps, then test the post-processing material for the presence of the contaminant. These studies will use rodent brain homogenate contaminated with scrapie as a model of CJD. They will employ inoculation of animals with the in-process material before and after the processing step to permit quantification of the step's ability to reduce or eliminate the contamination from the final product.

3. **Potential Transmission Risk.** Uncertainty exists regarding whether blood or plasma products may be vectors for transmission of TSE diseases. Although epidemiological evidence to date does not suggest transmission has occurred (L. Schonberger, 1997), some animal studies have suggested this possibility. Efforts to supplement the scientific understanding of the potential that blood and plasma products have for transmission of TSE diseases demands carefully designed and controlled studies. Bayer is conducting and collaboratively supporting ongoing study of this question. By analogy with viral infectivity, quantifying maximum possible levels of contamination in blood or plasma pools (should it exist) will define the clearance requirements for processing steps. These studies will employ both

infectivity and *in vitro* assay methodologies (as they become available through the above research efforts) and are hoped to provide a better understanding of the risks and safety of plasma products from transmission of TSE diseases.

While completion of this research represents a long-term but high priority commitment to the scientific study of CJD, the company expects interim results from the initial clearance and transmission studies before the end of 1997 and plans to review them at the NIH/FDA workshop in September of this year. These findings will not only guide the direction and scope of subsequent activities, but also may lead to a greater scientific understanding of TSE. Our immediate research goal is to determine:

◊ whether blood or plasma products are vectors for transmission of these types of diseases

◊ if reliable quantitative analytical procedures can be developed; and

◊ how treatment processes could remove any infectivity that may evade the company's pre-process screening systems.

Bayer is making a significant and increasing commitment to this research. We will share our findings with the scientific community at large in the hope of both advancing our common knowledge of TSE disease and whether plasma products pose any risks for its transmission.

A COMMENT ON PLASMA POOL REGULATIONS

Mr. Chairman, the Subcommittee has asked Bayer to provide views on regulating the size of plasma pools, or what I will call donor exposure in a final container of product (that is, how many individual donors contribute to the batch which results in a final lot of product). While it is debatable to what degree reducing donor exposure contributes to the ultimate safety of plasma products and whether the costs associated with severe restrictions in donor exposure are justified relative to the benefit obtained (especially in light of other measures which can contribute far more towards product safety), Bayer agrees and supports the commitment expressed by the industry association to reduce donor exposure. Indeed, for most products Bayer's current practice is significantly lower than this minimum commitment.

We share the spirit of those seeking to reduce donor exposure where safe and practicable. We will continue to strive to reduce exposure in all of our products. However, reducing donor exposure to help the patients who depend on plasma products is a highly complex question. I will illustrate this point with the example of a product Bayer makes for people with a disorder known as alpha$_1$ antitrypsin deficiency, or A_1AD, although this example is applicable to a number of protein products.

Alpha$_1$ antitrypsin (AAT) is a protein produced in the liver that protects the lungs from excessive action of a natural enzyme called neutrophil elastase. A lack of adequate AAT is associated with pulmonary destruction and severe emphysema in adults. The FDA approved Prolastin® (the Bayer tradename for AAT) as a replacement therapy for this hereditary disease in 1987, developed with the support of the Orphan Drug Act.

Patients undergoing this therapy must receive Prolastin® intravenously on a regular (weekly) basis throughout their lives in order to maintain normal circulating levels of this protein. Thus the health of A_1AD patients depends not only on this product's safety but also on its availability. Access to Prolastin® has been a problem: at present, Bayer (the sole producer) cannot meet the needs of all A_1AD patients. To assure that Prolastin® is available to as many patients as possible, we now require about 60,000 donor exposures per final lot of product. There are several reasons for this:

> *First*, a unit of human plasma contains only a small fraction of AAT. An average plasmapheresis donation, three-fourths of a liter of plasma, provides about one-fourth of a vial of AAT. Each patient requires about four vials per week. Assuming there are about 75 people in this hearing room, if we all donated plasma today, we could provide only a one month supply of this treatment for one patient.
>
> *Second*, scarce resources dictate efficient processing. Bayer relies on economies of scale gained through large pool fractionation to efficiently obtain as much product from source plasma as possible. In the manufacture of plasma products, the combination of specific intermediate fractions from different pools of plasma is necessary to achieve a sufficient quantity to enable efficient, cost effective processing. This is important for products like Prolastin® which are only present in very small quantities in plasma.

Currently, the freeze-drying equipment used in the final processing of Prolastin® limits our ability to reduce donor exposure: filling the capacity of these units (which normally run 24 hours a day, 7 days a week) permits the maximum amount of product to be made, but can result in a batch size of up to 60,000 donor exposures per final container lot. We could reduce the batch size by only partially filling the freeze-dryer; however, this would directly translate to reduced product availability since we have no idle freeze-dryer capacity at this time. We estimate that by immediately imposing a 15,000 donor exposure limit, we would reduce our ability to provide this life-giving drug by as much as 50%, as well as impacting supply of other products which utilize the same freeze dryers (e.g., AHF).

With additional freeze-dryers, we can reduce donor exposure to about 15,000 donors per lot without cutting the product lifeline to A_1AD patients. However, plasma fractionation requires custom-built equipment, as well as validation and FDA approval of all changes in manufacturing procedures, a process our past experience predicts could take several years to accomplish. Meanwhile, we are working aggressively to improve our manufacturing processes so that more product can be obtained from a unit of plasma. Investing in improved processing technologies that result in improved yields has the same end result as adding redundant equipment. By increasing the amount of final product made from a unit of plasma we effectively limit the donor exposure per product lot under the scenario described above.

From this example we wish to illustrate how reducing donor exposure needs to be considered in light of life-saving product availability, time and resources required to attain significant reductions and alternatives which have the potential of achieving similar ends. As one considers even more substantial reductions in donor exposure (for example, to 5,000 donors per lot) this equation becomes all the more complex. Multiple processing steps would be impacted, requiring in many cases construction of new facilities and the development of new technologies to accommodate the changes in scale and efficiency. It should be clear from the above example that this would be an expensive, complex and substantial undertaking for many products with time lines that stretch well into the 21st century.

We believe that a rational approach towards improving plasma product safety which integrates both effective material management to reduce donor exposure and the introduction of new process technologies affords the most reasonable opportunity to achieve our common goal of reducing safety risks to the patients who depend upon these life-saving therapies.

A COMMENT ON PRODUCT RECALL EFFICIENCY

Mr. Chairman, the Subcommittee has asked us to provide views on product recall efficiency. Bayer has contributed to and supports the proposal presented by industry representatives at the March 13, 1997 Blood Products Advisory Committee meeting. We believe that the industry's proposal to establish a well-publicized Internet web-page for communication of these matters is a quickly achievable first step and support its development. While endorsing this proposal for its practical approach in using existing systems to accomplish a responsible notification to patients, we also wish to remain in active dialogue with patient organizations, physicians, the FDA and the Subcommittee on this matter.

By example, in the years 1996-7, Bayer has had 9 withdrawals or recalls of plasma products; of these, 7 have been due to the post-manufacture discovery that one or more donors were identified as being at risk for infection with CJD. As a leading supplier of plasma-based products, Bayer recognizes there will always be some risk that a pathogen may evade even the most rigorous supply screening system. Bayer must be alert and respond quickly to issues of plasma safety, based on the unique circumstances of each situation.

A COMMENT ON SALINE DILUTION OF PLASMA SAMPLES

Mr. Chairman, your staff asked for comments on preventing testing errors caused by saline dilution of plasma samples. Bayer obtains plasma through a network of more than 130 plasma collection centers in the United States. Some plasma centers are owned and operated by Bayer, others are contract centers. Each center is licensed and inspected by the US Food and Drug Administration, and also regulated by state authorities. In 1989, Bayer converted all of its centers to Haemonetics PCS autopheresis machines and a no-saline protocol. Saline is used only when a donor requires it, which occurs very infrequently. Recently, two Bayer centers converted to redesigned Baxter Autopheresis-C (version 2.9a) devices, equipment that makes it nearly impossible to dilute a sample with saline. This newer version of Baxter equipment is also used at five of Bayer's contract plasma centers. The remaining contract centers use Haemonetics PCS devices. Thus, Bayer is using equipment designed to prevent saline dilution throughout its plasma collection operations.

A COMMENT ON THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES REPORT ON HIV ANTIBODY PREVALENCE RATES

Mr. Chairman, your staff asked us to comment on the report issued in December of 1996 from the California Department of Health Services which reported on the incidence of positive HIV antibody tests from donations of blood or plasma at either blood banks or plasmapheresis centers.

This is a very complex matter since the precise definitions, statistical methodology and reporting requirements are essential to understand to assure comparisons are accurate: for example, are self-exclusion criteria considered before or after the donor is included in the study? Are repeat donors only considered, particularly in light of the industry-wide initiative to implement an "applicant donor" program? Are the statistical samples accurately matched for variables (geography and socio-economics characteristics, etc.) that can influence endemic viral infection rates?

We believe a fair and scientifically sound discussion of viral marker rates is required to interpret the significance of this or any such comparative study and would prefer to defer comment until after this has occurred.

CONCLUSION

Bayer takes to heart its commitment to be a good corporate citizen. I reiterate the pride Bayer takes in its commitment to research excellence, its conscientious approach to producing quality products, and the openness it takes in addressing the challenges as a supplier of life-saving and life-enhancing biological products. As you have seen in this testimony, since the last time I have addressed this subcommittee, Bayer has undertaken numerous initiatives to potentially improve the safety of our plasma products, and has numerous activities afoot.

We believe there are four important areas in which we can, and do, contribute to safety and continued improvements in patient care:

First, screening of plasma donors, inventory hold and testing of plasma prior to manufacturing.

Second, continuing improvements in manufacturing processes and viral inactivation and clearance technologies.

Third, swift and responsible action in the event a potential pathogen is discovered.

Fourth, research and development into new treatments.

In consideration of seeking the most results-oriented means to improve plasma product safety measures, Bayer would encourage that a comprehensive orientation be taken in addressing individual approaches affecting safety outcomes. As stated previously, Bayer is committed to reducing donor exposure, but only as part of a more thorough approach to addressing safety improvement. That approach should include not only effective materials management, but also donor selection initiatives, inventory hold measures, PCR and other infectivity measures, as well as rigorous research programs into new areas of concern, such as CJD. We hope that this approach and understanding will be shared by this committee, the FDA, patient groups and all who are concerned with the well-being of the patients whose lives depend upon plasma products.

To see first hand how Bayer is applying a balance of measures in the service of plasma safety, I invite the Subcommittee members and staff to tour Bayer's fractionation facility near Raleigh, North Carolina.

Thank you again for the opportunity to speak today. I would be pleased to respond to any questions you or the committee may have.

Mr. SHAYS. Dr. Gomperts. Am I saying your name correctly?
Mr. GOMPERTS. Yes, you have.
Mr. SHAYS. Thank you, sir.
Doctor.

Dr. GOMPERTS. Mr. Chairman, members of the committee, I'm Dr. Edward Gomperts. I am the medical director of the Hyland Division of Baxter Healthcare Corp. In addition, I'm on staff in the Division of Hematology Oncology at the Children's Hospital of Los Angeles.

Baxter firmly believes that the task of ensuring that our patients have access to the highest quality therapies requires that the entire system of producing these therapies be subject to continuous improvement and critical examination. No one aspect of the process alone can ensure safety. That is why Baxter supports research and development focused on new processes for viral inactivation, and replacing donated proteins with synthesized proteins, and on developing cures for inherited conditions.

In particular, through our Aegis project, Baxter has recruited a panel of world-class scientists to work with our staff to scout out emerging pathogens and devise strategies to prevent introduction of such pathogens into our products.

In today's discussion, I would like to focus my comments on three areas: safety, pool size, and Creutzfeldt-Jakob disease. Let me begin by saying that plasma derived therapeutics are safe. However, speaking as a physician, the reality is no useful medicine is without risk.

We at Baxter focus on patient welfare and strive to achieve maximum safety and efficacy for our products. We have a comprehensive safety and quality assurance program, which is detailed in my written testimony. We try, we learn, but, at times, we make mistakes. When we err, our staff, FDA, our competitors and this committee and outside critics keep our attention on the target of patient welfare.

Before changing our well-established processes, however, we must guard against making choices for the wrong reasons. Science, reason, and patient welfare should dictate what we do, not political pressure, not media scrutiny, not a desire for profit.

Baxter has been asked to re-examine its production processes and determine whether the number of donors per pool has a relationship to safety. This is a very complex issue. Baxter agrees to take steps as outlined in my written testimony to decrease the total number of donors who contribute to the finished product. We believe, however, that pool size reduction is not a panacea. Moreover, there is a compelling need for these therapies.

Currently, our facilities are operating 7 days a week, 24 hours per day, and there is still unmet demand for certain products. Even under the most favorable conditions, additional manufacturing facilities take upwards of 3 to 5 years before they are permitted to make a meaningful contribution to overall supply.

An international crisis resulting in conflict or a major disaster could result in demand exceeding the current supply. Therefore, we want to caution against ill-advised decisions on manufacturing techniques which could skew product supply and alter safety and

efficacy needed by one group of patients to the detriment of another set.

In reducing pool size, we anticipate working closely with the FDA to develop and implement a strategy that would allow us to appropriately implement practical pool size limitations which will not undermine the important objective of ensuring access to an adequate supply of plasma-based therapies.

Now, let me turn to the issue of CJD. Extensive worldwide analysis of the transmission of sporadic CJD and its variant continues to point toward food contamination, and the ingestion of contaminated material is the key medium of transmission for a yet-to-be-identified infectious agent. In contrast, epidemiologic studies, ongoing and completed, have not today identified a blood mediated transmission mechanism. Unfortunately, I fear that the theoretical risk of blood transmission is receiving more attention in this country than the documented potential for food contamination.

An understanding of the potential risk of blood transmission will require completed, analyzed, peer-reviewed data for multiple carefully conducted and appropriately controlled animal studies. Through our Aegis project, my own company is advanced in the design of a research study to be carried out on primates as well as mice. Yet, in these studies, time is a serious problem. From development of a scientifically valid research plan to final results takes years.

In summary, I would like to affirm that Baxter utilizes the best scientific research and newest technologies to develop and improve our therapies and products for saving and enhancing patients' lives worldwide. We follow a policy of critical examination of our processes and continuous improvement. We remain committed to an open dialog with patients, treaters, the FDA, and Congress on our responses to scientific and medical changes. Thank you.

Mr. SHAYS. Thank you, Dr. Gomperts. I appreciate both your testimony and the previous testimony being within the 5-minute limit. Thank you very much. It's very helpful testimony.

[The prepared statement of Dr. Gomperts follows:]

Summary

Good Morning. My name is Dr. Edward Gomperts. I am the Medical Director and Vice President of Medical Affairs and Clinical Development of the Hyland Division of Baxter Healthcare Corporation. In addition, I am on staff in the Division of Hematology Oncology of the Children's Hospital of Los Angeles.

In my testimony today, I want to leave you with four overall points:

- Baxter is committed to producing adequate supplies of the safest, highest quality plasma therapies in the world. Our therapies include coagulation concentrates used to treat bleeding episodes suffered by individuals with hemophilia; intravenous gamma globulin needed by persons, such as cancer patients with immune deficiency; and albumin which is administered to burn and trauma victims in hospitals all across the country.

- We carry out an extensive program of safety and quality management in all aspects of plasma gathering, processing, manufacturing, and post-marketing surveillance to ensure that our products meet our high standards of quality and excellence.

- While we believe that this program already assures quality, we are now instituting a series of steps that will further limit possible risks associated with large pools of plasma, and we will do so in a way that does not dramatically reduce the supply of life-saving products.

- Finally, we wish to add a caution: Restricting pool size is not a panacea. Limiting the number of donors may have untoward effects that we cannot now identify. In addition, restrictions in one phase of the complex process of developing plasma-based therapies might have harmful, though unintended, consequences in other areas.

Our goal, Mr. Chairman, is to protect consumers and to provide them with quality products in which they can have total confidence. Baxter's current products meet that standard. But we are also committed to doing whatever we can to advance the frontiers of quality in all aspects of our research, product development, manufacturing, and operations.

Background

Before providing the details of those views, let me introduce our company. Baxter Healthcare Corporation is the principal domestic subsidiary of Baxter International Inc. Through its subsidiaries, Baxter is the leading manufacturer and marketer of health care services and products -- including those derived from human plasma -- benefiting individuals in nearly 100 countries worldwide. It focuses its research and development programs on biotechnology, cardiovascular medicine, renal therapy, and transfusion medicine.

Recently, Immuno International became part of the Baxter family of companies, and the combination of the two organizations will continue the long record of scientific and medical leadership that each has compiled.

I am pleased to have the opportunity to provide the Subcommittee with an update of the progress that our industry generally -- and our newly combined companies specifically -- have made in providing patients in this country and around the world with an adequate supply of technologically innovative, effective, and therapeutic proteins.

Baxter Focuses on System-Wide Quality

Let me begin by saying that plasma derived therapeutics are safe. However, speaking as a physician, the reality is that no useful medicine is without risk. The only way to guarantee 100 percent safety of blood or its components is not to use them.

We at Baxter strive to achieve maximum safety and efficacy for our products, and we focus on patient welfare. We try, we learn, but at times we make mistakes. The FDA, our competitors, this Subcommittee, and outside critics help keep our eyes on the target of patient welfare. Before changing well-established processes, however, we must guard against making choices for the wrong reasons. Science, reason, and patient welfare should dictate what we do, not political pressure, not media scrutiny, and not a desire for profit.

Baxter firmly believes that ensuring patient access to the highest quality plasma protein therapies requires that the entire system of producing these therapies be subject to continuous improvement, as well as to careful and constant examination. We believe this will let us be absolutely certain we are doing the most that human knowledge allows to ensure both safety and quality.

For that reason, Baxter carries out a comprehensive quality and safety initiative (which I will highlight later in my testimony) in all phases of plasma gathering and product development -- including selection of the donor, processing of the plasma, inactivation of enveloped viruses, and post-marketing surveillance of the therapies we produce.

In real-world terms, that means our products are safe -- as safe as any medicine can be -- and that consumers can use them with confidence.

But we are also committed to finding even better answers for the future. For that reason, our quality program also includes development of entirely new technologies. Baxter is currently participating in research and development of technologies designed to provide further steps to inactivate viruses even before plasma is pooled, replace donated proteins with synthesized proteins, and provide genetic solutions to inherited conditions.

All of these efforts, together, reflect our view that true quality emerges not from a single factor, but from a comprehensive program that focuses on improvements in all areas. We believe this is a key point to keep in mind as the Subcommittee considers the question of pool size and its relationship to quality and safety.

Baxter Takes Steps to Reduce Donor Pools

On that issue, Mr. Chairman, let me say that we understand the concerns expressed by this Subcommittee and by the FDA about the size of plasma pools used to develop plasma derivative therapies. We take these concerns very seriously and appreciate the opportunity to discuss them with you today.

Baxter has re-examined its production processes and continues to debate whether the number of donors per pool has a significant relationship to safety. Frankly, we see no evidence to indicate that there is a safety issue linked to pool size, but we have no clear answer. Nevertheless, as a result of inquiries from the Subcommittee and the FDA and because the optimal number of donors in a pool remains an open question, we are taking a number of steps to reduce the size of the donor pools we use.

These are steps that can be implemented in short order, without lengthy review by FDA or massive redesign of our manufacturing process and facilities. Just as importantly, these are steps that will also allow the production of an adequate

supply of the full range of therapeutics without threatening their potency or stability.

The steps we will implement include the following:

- Baxter will no longer re-pool -- or combine -- small quantities of plasma material from different production runs. We have done this in the past to conserve and ensure maximum utilization of these life-saving therapeutic proteins.

- We will substantially reduce repooling of small quantities of plasma material from various production runs that had been rejected because of problems with their packaging. Note that these materials were not rejected because of concerns over safety, but because of such things as misaligned labels. Nevertheless, this change is useful in that it will allow us to maintain the identity of the plasma pool and track donors more easily.

- We will revamp our production procedures to draw the active protein for a specific therapy and the albumin we use to stabilize that protein from the very same plasma pool. Our target is to do this in at least 85 percent of the production. This will, in effect, reduce the number of donors in the pool for these products.

- We will adjust our inventory and process control procedures to set an upper limit on pool size -- as measured by the number of individuals donating units to the pool. This will be done on a per-product basis and will maintain the identity of the pool through all stages of production. (This step is consistent with the proposed industry standard set forth by the International Plasma Products Industry Association.) We will ensure that no final product exceeds the donor exposure cap and many final products will fall significantly below.

  This initial target will result in an immediate reduction in potential donor pool size on the order of 40 percent for some products. The remainder of our products already fall at or below those levels. In addition, we will periodically evaluate and reduce these levels as manufacturing technology, clinical safety data, and regulatory considerations permit.

Cautions About Inappropriate Reductions in Pool Size

After having summarized these actions, Mr. Chairman, I believe it is also important to add a couple of caveats with regard to limiting pool size.

First, we must be cautious about making radical changes. Our current manufacturing process was designed, validated, and licensed only after FDA reviewed our technical, scientific, and clinical data to be certain that the resulting therapies would be safe and effective. We were not permitted to distribute therapies from these facilities until we were able to generate data that convinced FDA that the resulting products met the standards of our license.

We do not now have the data on how radical changes in processing procedures might affect the safety or efficacy. Major changes in the process -- without FDA review and approval and without the supporting clinical data -- are not permitted, nor should they be. Because we are dealing in rare and fragile protein molecules -- which under certain circumstances can be rendered harmful to the patient -- we must avoid introducing unresearched and untested processing techniques.

Another caution to keep in mind as Congress contemplates limits on pool size is that there is a compelling human need for these therapies. Currently, Baxter facilities are operating seven days a week, 24 hours per day, and there is still unmet demand for some of our products. An international conflict that results in substantial loss of life or a major disaster could exhaust the current supply. Even under the most favorable conditions, building and licensing additional manufacturing facilities take upwards of three to five years before such facilities are capable of making, and are licensed to make, a meaningful contribution to the supply.

The point I am making is this: We must be certain that any efforts to limit pool size do not, inadvertently, increase danger for patients because they yield too little material to manufacture these life-saving therapies. Nor do we want decisions on manufacturing techniques to skew the product supply, safety, or efficacy needed by one group of patients to the detriment of another group. For these reasons, we look forward to working with the FDA to develop and implement a strategy that will allow us to continue to maintain practical pool size limits that will not undermine the equally important goals of ensuring access to an adequate supply of such therapies.

In this same context, Mr. Chairman, I believe a word must be said about the complexity of the process used to develop blood-based therapies and how that complexity factors into the questions of pool size and possible limits on pool size. In addressing this point, I would like to provide just a bit of background about how the process of developing these therapies works.

As you may know, the basic materials used to develop these therapies are found in blood -- more specifically, in blood plasma. Once blood has been collected and the plasma has been separated, step-by-step we add progressively increasing concentrations of alcohol to the plasma to separate out the individual proteins -- or *fractions* -- of the plasma, such as albumin, gamma globulin, and clotting factor concentrates. This process is known as *fractionation.* We then use these proteins in producing our therapeutics.

Fractionation is a delicate and complex process. Proteins are separated in a specific sequence by varying such factors as temperature, acidity, speed of the centrifuge, and so on.

This is generally how the process works. The plasma material is mixed in a tank and is then processed through the centrifuges at a specific temperature, alcohol concentration, speed, and pH so that a specific protein can be collected. The remaining material is then reprocessed by adjusting the characteristics of the solution and centrifuged again to separate out a different protein. As minute changes of this type are made, each of the targeted plasma protein fractions is collected for final processing to constitute the licensed therapeutic.

The challenge for the fractionators -- those who perform this process -- is to separate the therapeutic proteins from the rest of the material, without altering the protein's beneficial properties, while concentrating them so that they can be administered to patients. But this is no easy task. These proteins are fragile and are often similar in structure to the substances from which they must be separated. Further, a slight variation one way or the other in a myriad of factors can result in a failed lot, a significant decrease in yield, and the loss of a significant amount of precious human blood components. Even worse is the potential to render a therapeutic protein ineffective or even harmful to the patient who receives it.

The point, simply, is that this process of fractionating plasma to obtain these important proteins is highly complex, comprised of many interlinked and interconnected steps. This process is unique to my company and the facilities in which it is performed, and this is true of the other fractionators as well. Major

changes cannot be readily introduced in one step of the process, either upstream or downstream, without possibly affecting -- potentially adversely -- other areas. Even changes to enhance perceived safety in gathering one particular protein may reduce or eliminate another therapeutic protein -- thus harming other groups of patients.

Therefore, I return to the point I made a moment ago. Wholesale or dramatic changes that are imposed on this process -- including improperly designed limits on pool size -- can often boomerang, creating even deeper problems. We urge that all potential changes considered by this Subcommittee or FDA are given detailed clinical review so that we fully understand their impact before they are introduced.

Other Factors Besides Pool Size Are Critical in Safety

As Congress and FDA contemplate the question of pool size, we also ask you to take into account another important point: That is, safety and quality in blood-based therapies do not depend on the size of the donor pool, with the exception of the intravenous immuno-globulin product where larger pool size is needed. As we have indicated, this may be a factor. But a number of other elements in the gathering, processing, manufacturing, and production of such therapies and the materials used to make them provide the current substantial margin of safety.

I am pleased to report that Baxter carries out a comprehensive program to ensure quality in virtually every stage of this process. These include the following:

- QPP qualification of plasma centers. These are operating standards voluntarily adopted by plasma center licensees that are over and above the requirements of the FDA.

- Rigorous screening of all donors, including direct questioning and confidential exclusion of units from donors whose backgrounds may be of concern or in doubt.

- Testing all source material for Hepatitis B, Hepatitis C, and ALT and excluding any potentially harmful plasma from the starting pool and destroying those units. (ALT is a liver enzyme test that is a general indicator of liver function.)

- HIV screening (ELISA) of every unit of plasma we receive, as well as excluding potentially harmful units from the starting pools and destroying them. This has occurred since 1985, including use of second and third generation HIV tests and combinations of tests.

- Special procedures -- called Lookback procedures -- to exclude from the starting plasma pool any units from donors who have subsequently tested positive on one of our screening tests.

- Comprehensive procedures to inactivate and remove viruses from the plasma. Baxter's Hyland Division introduced the first licensed, viral inactivated factor concentrate in 1983. This featured a heat method of virus inactivation. Subsequent refinements and additional technologies include monoclonal antibody affinity purification procedures, as well as other viral inactivation procedures, including solvent detergent treatment and vapor heating.

- Experiments being conducted to validate careful PCR screening of plasma pools to be processed and PCR testing of all batches of our therapies before they are released. PCR is a test that identifies certain sequences in the nucleic acids of specific viruses, rather than identifying antibodies that the human body prepares to battle the viruses. (Such antibodies are an indirect indicator of viral presences.)

In addition to these initiatives underway at Baxter, Mr. Chairman, I also want to outline the extensive quality system that has been in place at Immuno. This program has included placement of plasma centers in areas with low rates of potential blood-borne viruses, efforts to encourage repeat visits by well-qualified donors, comprehensive quality control auditing procedures, exclusion of questionable donors (including one-time donors), and special procedures to hold units in inventory longer, thus increasing the chances further that any potentially contaminated units of plasma will be detected and destroyed. On July 1, the members of International Plasma Products Industry Association (IPPIA) and American Blood Resources Association (ABRA) adopted a new voluntary standard which implements key aspects of this program, particularly the one-time donor exclusion, extended inventory holds, and PCR testing. These procedures are being adopted as rapidly as possible throughout the Baxter system and the entire industry.

The results of these quality-control procedures, when fully implemented, are dramatic. Data from Community Bio-Resources, Inc. (CBR), an early adopter of these procedures and now a part of the Baxter family of companies, demonstrate the improvement in the margin of safety for plasma products. CBR plasma processed by Immuno showed viral reactive rates (per 100,000 donations) for the qualified donors for calendar year 1996 to be:

Viral Marker		Rate per 100,000 Units
(1)	HIV	0.26
(2)	HBV (hepatitis B virus)	0.66
(3)	HCV (hepatitis C virus)	1.05

In plain English, that means we found about one unit or less that carried these viruses in every 100,000 units of blood that were donated. Additional PCR testing of the last one million donations in the CBR plasma pools confirmed this low viral reactivity rate.

Keep in mind that this is even before further steps are taken to inactivate and remove viruses as the fractionation and production processes proceed. When the effect of these combined efforts are added together, it results in a very wide safety margin. Baxter and our entire industry are moving rapidly to implement this program through our systems.

In a nutshell, all of the statistics I've just cited mean that the plasma we use and the therapies we produce from that plasma are remarkably safe -- as safe as any medicine of any kind can be. As the Subcommittee contemplates the question of safety of blood-based therapies, we ask you to keep these results in mind. And we ask you to recognize, once more, that pool size issues must be considered in the context of the entire process of safety control, monitoring, and process management that we employ.

Baxter Views on CJD and Postmarket Surveillance

As part of our overall efforts to maintain quality and safety, post-market surveillance and recall of possibly unsafe product obviously play a key role. Let me briefly touch on those topics, before turning to questions related in particular to CJD.

Each of the recalls that Baxter has carried out in the past has adhered to current regulations and has focused on the safety of the end-user, the patient. I believe our targets have been successfully accomplished, but -- as in all areas -- we seek to continually improve the process and are open to all meaningful and practical modifications or additions. We have been challenged by Dr. Pendergast of FDA to work together with the representatives of consumer and patient groups, and these interactions are taking place.

As you know, Mr. Chairman, the topic of recalls certainly relates to CJD and concerns over its possible presence in the blood supply. Because of those concerns, we have carried out five separate recalls so far this year because a plasma donor may have previously used autologous dura mater or received injections of human growth hormone. In each of these, we have sedulously adhered to the requirements for such recalls at all levels. Nevertheless, this issue has caused enormous consternation and fear among numerous users and beneficiaries of albumin, where the possibility of transmission of CJD is too low to be determined.

Ongoing epidemiologic analysis of sporadic CJD and its variant, both in the U.S. and Europe, continues to point towards food contamination and the ingestion of contaminated material as the key medium for transmission of a yet-to-be-identified infectious agent. In contrast, epidemiologic studies, both ongoing and completed, have not identified a blood mediated transmission mechanism to date. Completed, analyzed, peer-reviewed data from carefully conducted and appropriately controlled animal studies has yet to take place on this issue.

My own company is advanced in the development of a research study to be carried out on primates as well as mice, but time is a serious problem since it takes many months to develop a meaningful research plan, months to initiate the plan properly, and years to obtain the results. However, I fear that the blood-transmission issue is receiving more attention in this country than the food contamination problem.

Baxter and Plasma Industry Are Conducting Wide-Ranging Research

One of the clear lessons of the CJD issue, Mr. Chairman, is that we can never be content with the current state of knowledge of disease or the current capacity for ensuring high quality and safety. We must continue to seek new techniques and new strategies for reducing risks, enhancing safety, and improving the quality of plasma protein therapies.

In effect, we must continue the progress that has occurred in hematology over the past 50 years. During that time, progress has been marked by utilizing technology to conserve the life-giving properties of blood and plasma and of administering ever more precisely the specific protein that the patient needs. In the 1920's and 30's, blood therapies consisted of whole blood or packed red cell transfusions. Then researchers discovered the ability to separate plasma from blood cells and, during World War II, to remove the albumin from the plasma. Still later, researchers discovered new fractionation techniques that allowed them to separate other proteins, such as coagulation concentrate and gamma globulin.

This entire process has rested on two very clear and very critical realities: First, therapeutic proteins come from the blood of ordinary citizens who are willing to give of themselves to aid their fellow citizens. These proteins are not found in isolation. Second, the progress we've seen of finding ever more precise proteins to better-fit the needs of patients relies upon this very complex, very essential process of fractionation that I described earlier -- as well as on the intensive research over the years that has increased our knowledge of this technique.

We are committed to the pursuit of continually improved techniques to continue that march toward safer, better protein therapeutics. All of this takes time and energy and effort, but those efforts have been worthwhile for they have borne clear improvements in safety and quality.

Now, Mr. Chairman, I would like to highlight a number of research areas that we believe are also critical steps for the future. Each of these initiatives offers the potential for enhanced safety and quality as they reduce risk.

A major Baxter initiative is Project Aegis. This is a comprehensive effort by Baxter's research staff and a panel of world class scientists to scout out potential threats of infections transmissible by blood or plasma, and to develop new technologies and techniques to detect, identify, and remove units of plasma possibly containing pathogens before they can enter the production process.

Its goals also include developing new techniques for viral inactivation or removal. Possible new approaches include nano filtration, a technique to filter out small viruses, and methods to inactivate small, non-enveloped viruses, which are highly resistant to our current inactivation technology. Various methods of photo-dynamic binding or destruction of viral and bacterial genomic nucleic acid are also being tried.

In addition to this research, Baxter is seeking solutions for tomorrow in a number of other areas:

- Consortium for Plasma Science

  Baxter has taken a leading position in the formation and operation of the Consortium for Plasma Science, whose sole purpose is to identify innovative research on new technology that would inactivate viruses. This industry-supported collaborative research organization has allocated substantial seed money -- $20 million over five to six years -- to promote and fund research and development of new technology in the inactivation of viruses and other pathogens that might affect blood and plasma. Additional resources will be committed as circumstances warrant. A Request For Proposal was published early this year and multiple research proposals in response have been received by the Consortium. These are currently under evaluation by the Consortium expert scientific review committee. Successful technology will be licensed to all member companies and will be offered to other applicants.

- Enhanced PCR Tests

  Immuno has taken a leading position in the development of highly sensitive PCR technology to screen all donated plasma for the major viral pathogens -- HIV, HCV, HBV, and additional viral markers. New systems will be introduced as rapidly as possible. Baxter and Immuno researchers and technical staff are working with the FDA, other fractionators, and the American National Red Cross to obtain regulatory approval for licensing this PCR technology to screen plasma for known blood-transmitted viral agents.

- Recombinant DNA Research

  In 1992, FDA approved Baxter's Recombinate, the first genetically engineered synthetic Factor VIII, which is used to treat the most common form of hemophilia. We are now conducting research on the use of recombinant DNA technology to synthesize additional plasma proteins -- thereby reducing the need to rely solely on human plasma for therapies to treat patients with a variety of inherited conditions.

- Blood Substitute

  Baxter is completing Phase III clinical trails on its virally inactivated, oxygen-carrying substitute for human hemoglobin. This will provide an alternative to whole blood or packed red cells in many treatment areas.

- Gene Therapy

  Baxter continues its research to find a genetic answer -- and hopefully a cure -- for inherited diseases such as hemophilia, thereby obviating the need for continued therapy. This is a potentially revolutionary approach with enormous potential. It would allow a physician to prescribe a treatment which -- instead of supplying the missing protein --would supply the needed gene so the patient's body could provide the needed proteins.

Conclusion

In conclusion, Mr. Chairman, let me reiterate that our company continues to be committed to making the best, safest, highest quality products. We are constantly seeking -- and are always open to -- suggestions that will achieve this in the most effective manner, both today and tomorrow.

But we believe that all efforts to achieve these goals must be realistic, must ensure an adequate supply of therapies for all patients, must recognize the extraordinarily complex process of developing blood-based therapies, and must be part of a comprehensive and long-range program that ensures quality today as it unlocks the mysteries of disease for tomorrow.

Baxter continues its goal of utilizing the best scientific research and the latest techniques to achieve quality and safety. We remain committed to a policy of critical examination of all processes we use, to continuous improvement of these processes, and to maintaining an open and frank dialogue with patients, doctors, FDA, and Congress as we respond to these scientific and medical challenges.

I thank you for the opportunity to share these views and developments with you.

Mr. SHAYS. Dr. Feldman.

Mr. FELDMAN. Is this OK?

Mr. SHAYS. I think it will do. Let's see how it sounds.

Mr. FELDMAN. Mr. Chairman, and members of the subcommittee, my name is Fred Feldman, and I'm vice president of Preclinical Research and Development for Centeon. I've dedicated my efforts for more than 20 years to the development of new and improved therapeutics from plasma, and I'm happy to contribute to the deliberations of this committee on the topic of pool sizes.

This is without doubt a highly specialized area. I have endeavored at every opportunity to assist several blood product advisory committees as well as the staff of this committee in understanding this area and hope that I can be of assistance today as well.

This is not a trivial manufacturing issue, and a decision to constrain manufacturing to a substantially reduced total pool volume can have the effect of reducing the total therapeutic product supply.

Mr. Chairman, in the interest of time, I ask that the full written testimony which I have provided be included in the record.

Mr. SHAYS. Yours will be, as we will as all the other witnesses.

Mr. FELDMAN. Thank you.

Appendix 1 to my full testimony provides a report describing the impact of plasma batch sizes, described in liters, on the manufacture of our Factor VIII Concentrate. It was motivated by reported concerns that to significantly enhance viral safety for chronic users, a very drastic reduction of process volumes and batch sizes would be needed, and it explored the impact of large reduction of process batch volumes.

The report shows that for such a reduction in batch size, that such a reduction greatly increases the complexity of production and places a greater burden on GMP and quality assurance, while reducing usable product from existing plants by huge amounts.

It can be seen in the poster of table 9 from this report that changing from production batches at 15,000 liter equivalents to 500 liter equivalents could decrease product supply over 96 percent. From the throughput capability of our plant, we could serve the needs of over 4,500 people per year with hemophilia A. We would be reduced to being able to serve the needs of only 160.

As an industry, however, our products serve a wide range of the public. We assessed the impact of batch size on the other products we manufacture using the Cohn fractionation method. Deliberations on risks and disadvantages of changing manufacturing of human albumin and human intravenous gammaglobulin are detailed in the second appendix to this report as well as opportunities for improvement in these processes.

As we have participated in and learned from this dialog, we must consider not only benefit for the chronic user, but also benefits which could result to the patient who only occasionally receives our therapy.

We believe that it is incumbent upon manufacturers of critical drugs not only to continue to supply product reliably, but to look for improvement opportunities. Although we believe that substantial changes to process volumes are difficult to achieve, potentially disruptive of supply, and even in instances frought with risk of re-

duced safety, we have asked where there are opportunities within the existing fractionation system, where improvements in control and donor exposure can be made without creating significant, regulatory, or supply disruptions, and where risk reduction benefit could result at least to that patient infrequently exposed to pooled plasma derivatives.

We have identified opportunities for Centeon to reduce the total donor exposure from a given batch and have initiated the nine point program of improvement shown on the poster. These specific initiatives can decrease the maximum number of donors in our processing and reduce the overall variation in the number of donors associated with any given batch of therapeutic. The specific process-by-process changes as well as the equipment-related opportunities which may offer other means for improvement will be reviewed with FDA to ensure compliance with cGMP and control of quality assurance before changes are initiated.

We would hope that the committee recognizes that such change, such validation, takes not only resources, but takes time, and that even seemingly trivial changes in production equipment require us to develop assurance that our processes remain in total control, that our changes do not impact the stability of our therapies, nor adversely impact their safety, efficacy, or availability to the patient community.

Let me conclude by saying that we continue to dedicate our R&D resources to understand where other threats to safety might originate and to design and implement yet further barriers to these threats, through increased surveillance using an outside panel of top worldwide experts, through working to develop and improve tests for donors and products, and through efforts to provide even more choices for powerful separation and inactivation method.

Mr. Chairman, thank you for allowing me to appear here today, and I'm happy to answer whatever questions I can from the committee.

Mr. SHAYS. Thank you very much, Dr. Feldman.

[The prepared statement of Mr. Feldman follows:]

Mr. Chairman and Members of the Subcommittee:

My name is Dr. Fred Feldman, and I am Vice President of Pre-Clinical Research and Development for Centeon. I have dedicated my efforts for more than 20 years to development of new and improved therapeutics from plasma, and I am happy to contribute to the deliberations of this committee on the topic of pool sizes. This is, without doubt, a highly specialized area. I have endeavored at every opportunity to assist several Blood Product Advisory Committees, as well as the staff of this committee in understanding this area and hope that I can be of assistance today as well. This is not a trivial manufacturing issue and a decision to constrain manufacturing to a substantially reduced total pool volume can have the effect of reducing the total therapeutic product supply. Mr. Chairman, in the interest of time, I ask that the full written testimony which I have provided be included in the record.

Appendix 1 to my full testimony provides a report describing the impact of plasma batch sizes (described in Liters) on the manufacture of our factor VIII concentrate. It was motivated by reported concerns that to

significantly enhance viral safety for chronic users, a very drastic reduction of process volumes and batch sizes would be needed, and it explored the impact of large reduction of process batch volumes. The report shows that such a reduction in batch size greatly increases the complexity of production and places a greater burden on GMP and quality assurance, while reducing useable product from existing plants by huge amounts. It can be seen in the poster of Table 9 from this report that changing from production batches at 15,000 Liter equivalents to 500 liter equivalents could decrease product supply over 96%. If from the throughput capability of our plant we could serve the needs of over 4,500 people per year with hemophilia A, we would be reduced to being able to serve the needs of only 160.

As an industry, however, our products serve a wide range of the public, and we assessed the impact of batch size on the other products we manufacture using the Cohn fractionation method. Deliberations on risks and disadvantages of changing manufacturing of human albumin and human intravenous gamma globulin are detailed in the second appendix to this report as well as opportunities for improvement in those processes.

As we have participated in and learned from this dialogue, we must consider not only benefits for the chronic user but also benefits which could result to the patient who only occasionally receives our therapy.

We believe that it is incumbent upon manufacturers of critical drugs not only to continue to supply product reliably, but to look for improvement opportunities. Although we believe that substantial changes to process volumes are difficult to achieve, potentially disruptive of supply, and even, in instances, fraught with risk of reduced safety, we have asked where there are opportunities within the existing fractionation system where improvements in control and donor exposure can be made without creating significant regulatory or supply disruptions and where risk reduction benefit could result, at least to that patient infrequently exposed to pooled plasma derivatives.

We have identified opportunities for Centeon to reduce the total donor exposure from a given batch and have initiated the nine point program of improvement (Appendix 2: Table 1) shown on the poster:

These specific initiatives can decrease the maximum number of donors in our processing and reduce the overall variation in the number of donors associated with any given batch of therapeutic. The specific process-by-process changes, as well as equipment related opportunities which may offer other means for improvement, will be reviewed with FDA to ensure compliance with cGMP and control of quality assurance before changes are initiated. We would hope the committee recognizes that such change, such validation, takes not only resources but takes time and that even seemingly trivial changes in production equipment require us to develop assurance that our processes remain in total control, that our changes do not impact the stability of our therapies nor adversely impact their safety, efficacy, and availability to the patient community.

Let me conclude by saying that we continue to dedicate our R&D resources to understand where other threats to safety might originate and to design and implement yet further barriers to these threats, through increased surveillance using an outside panel of top world-wide experts, through working to develop improved tests for donors and products, and through

efforts to provide even more choices for powerful separation and inactivation methods.

Thank you, and I am happy to answer whatever questions I can from the committee.

Table 9: Batch Size vs. Product Availability
Monoclate-P (Factor VIII)

B: (With Albumin Stabilizer Donors According To Current Practice)

Liters Per Batch:	15,000	5,000	1,500	500
Donors Per Batch Range:	53,000-81,000.	47,000-70,000.	43,000-65,000.	42,000-64,000.
Batches Per Week:	3	5	5	5
Vials Per Batch:	2250	750	225	75
Weeks Per Year:	52	52	52	52
% Net vials Released:	98	94.6	82	61
Potency (Units Per Vial):	1,000	1,000	1,000	1,000
Million Units Produced Per Year:	344	185	48	12
Patients Served Per Year:	4,587	2,467	640	160

Table 2: Donor Pool Improvement Program

1 Identify And Decrease The Total Donor Exposure Range

2 Eliminate Add-back Of Recovered Units

3 Reduce Combined Sections On Factor VIII

4 Limit Addition Of Stabilizers To A Single Lot At A Time

5 Make A Special Small Lot Of Stabilizer For Use In Factor VIII

6 Reduce The Pool For Beginning Factor IX Production

7 Limit The Cryo Poor Pool For Cohn Process To Two Sections

8 Combine Only Two Fr II+III Pastes For IVIG

9 Control Repeat Donor Rate To Gain 50% Repeat Donors In Pool

Appendix 1

Impact of Plasma Batch Size On Manufacturing Of Coagulation Factor Concentrate

APPENDIX 1

IMPACT OF PLASMA BATCH SIZE ON MANUFACTURING OF COAGULATION FACTOR CONCENTRATES

Understanding the molecular defects causing hemophilia bleeding disorders has been a slow process. Although the genetic predisposition of the disorder was suspected even in biblical times, the disease and its treatment was poorly understood even until the early 1950's. As it became understood that hemophilia A could be corrected by replacement of plasma coagulation Factor VIII, early treatment took the form of replacement therapy with whole plasma. Low concentrations of the factor and large volumes needed for treatment limited this to the most urgent conditions. Improvements in the 1960's[1],[2] and 1970's[3] led to the development of highly concentrated manufactured concentrates which enabled aggressive treatment of severe bleeding conditions, enabled home care and prophylactic treatment to prevent joint disorders and enabled the prevention of life-threatening spontaneous bleeds.

The undetected contamination of the blood and plasma supply by the AIDS virus took a tragic toll before the virus was identified by scientists and aggressive virus inactivation and removal methods could be introduced during

the 1980's. Increasing understanding of the structure and biochemistry of Factor VIII[4] coupled with the availability of more sophisticated separation methods enabled the preparation of the first monoclonal antibody purified Factor VIII concentrates in the mid 1980's[5] (licensed first in 1987). Several techniques for inactivating viruses are now in use in the preparation of Factor VIII concentrates world-wide including the use of solvent/detergents for inactivation of lipid-enveloped viruses[6] and the use of various heating regimens, both in the liquid[7],[8] as well as in the lyophilized product state[9]. High orders of virus removal have been documented for many such concentrates dramatically improving their safety[10],[11],[12]. During the 1990's recombinant Factor VIII concentrates have also become available, reducing dependence on plasma sourcing, although these products all require stabilization with large quantities of human plasma derived albumin. Generally, the supply of Factor VIII globally, however, is still greatly dependent on the availability of plasma derived Factor VIII.

In recent meetings, questions have been raised with regard to the necessity for large scale processing of plasma therapeutics. It has been presumed that reducing the number of donors that contribute to a batch of product and reducing the size of the starting pool for fractionation will significantly

improve the safety of the resultant product. For patient groups requiring ongoing continuous access to such therapeutics, such an assumption requires a more comprehensive evaluation. As product usage increases, either by exposure to multiple donors from a single large batch or by exposures to multiple donors by use of many small batches, the risk to the chronic user rises similarly. It has been estimated by Woods and Horowitz[13] that until donor pools are significantly less than 100 donors (approximately 75 liters of plasma) that the accumulated risk to the long-term chronic user is little improved over product obtained from large pools.

It can be shown that one of the impacts of limiting production to small pool sizes would be to drastically reduce the total available product which could be produced on a national basis. Since a very significant portion of world-wide fractionation capacity resides in the United States, mandated reduction to very small pool sizes for fractionation would reduce product availability not only in the United States but world-wide and create difficulties in product availability for treatment of a large range of disorders - hemophilia, immune deficiency, RH incompatibility, auto-immune disorders, alpha-1-protease deficiency, anti-thrombin III deficiency, treatments of burn and trauma patients requiring transfusions with human albumin, treatment of burn and

accident victims requiring use of fibrin sealants for trauma or burns (approved in Europe and Japan).

For purposes of this discussion, we illustrate in detail the impact of reducing batch size of a generalized Factor VIII produced using first cryoprecipitation, then immuno-affinity chromatography followed by secondary chromatography. A generalized recovery of 150 units Factor VIII per liter plasma fractionated is assumed, a value well within the types of recoveries found by others in a variety of fractionation methods. Variation of yields outside this range has little to no effect on the conclusions reached and can be interpolated from the data provided.

Table 1 demonstrates what happens to process scale as a function of batch size and increasing purification. The first column demonstrates the various process steps employed in purification/processing. Three different total process batch volumes are explored throughout this evaluation: 15,000 liters, 1500 liters, and 500 liters. It is assumed that in any of these process volumes that five subsections are prepared individually to an intermediate holding point and, as process volume is reduced below a practical handling scale, that subsections are combined to a single master lot (15,000 liters, 1500 liters, or

500 liters). Table 1 shows that even when a process is begun with a large volume of 3,000 liters, that by the time the intermediate holding step is reached, the volume is reduced to 7.5 liters. Combination of five such sections (thereby making the equivalent batch volume now equal to 15,000 liters) raises the in-process volume to 37.5 liters for continued processing. Even by this combination, the final sterile bulk product attained for filling into vials is only 9 liters. When filled into vials of 1000 unit per vial potency 2,250 gross vials are produced. This number is further reduced by sampling requirements and quality control testing needs.

It can be seen in like fashion from table 1 what happens as starting batch volume is reduced. Reducing the initial batch from 15,000 liters to 1500 liters results in process volumes at the end of processing of about 0.9 liters, a volume difficult to handle and still maintain the process in control.

If the initial process volume were reduced further from 15,000 liters to 500 liters, the situation becomes even more difficult. Volumes for processing are attained of only 0.3 liters. Such a volume makes process monitoring and control practically impossible and results in major product losses by coating of vessels. At 500 liters process volume, a volume that still does not

significantly reduce exposure to multiple donors, only 75 gross vials per product lot would be available for testing and release.

The practical impact on attempting to operate production systems at the reduced volume scales described above is described in table 2. At 15,000 liters equivalent plasma pool size, it can be seen that the final bulk volume is 9,000 ml and that 2,250 gross vials are attained. Three such batches are presumed to be routinely processed in the course of a week, resulting in a yearly production capacity of 156 batches produced for distribution. Such production can be accomplished by filling vials in a single dedicated filling room facility. Additionally, such production requires the use of two large freeze-dryers. The freeze-dryers contain multiple shelves, but the contents of a single lyophilizer chamber are defined as constituting a single batch. Several different batches in a lyophilizer are not allowed as a violation of GMP (Good Manufacturing Practice). To conduct manufacturing at this scale, process control general requires 950 separate in-process product quality control tests per week and 63 quality control tests on final product per week. The quantity of product consumed in final product testing alone is about 2% at this scale.

The drastic impact on reducing plasma batch volume from 15,000 liters to 500 liters can be readily seen on Table 2. At this scale, a final bulk volume of only 300 ml is obtained, which yields only 75 gross vials of 1000 units per vial. To generate an equivalent number of gross units as production at 15,000 liter pool size, 90 batches per week would have to be produced at this small scale or as much as 4,680 batches per year. Since a lyophilizer can only contain a single batch of product, the number of lyophilizer chambers required would also increase dramatically. Rather than estimate 90 chambers required for drying, we have assumed optimization of the drying cycle to reduce cycle time, but believe that upwards of 60 industrial freeze dryers would still be required. Since a filling line requires dedicated equipment and stringent sanitization/qualification between batches, upward of 18 fill rooms and independent equipment would be required. The maintenance, qualification, and validation/revalidation of such facilities would further increase complexity. The number of in-process QC tests required for manufacture would increase to 6,750 per week and final product QC release tests would climb to 1890 tests per week. Data acquisition and control would be a significant factor as would availability of equipment and facilities. Lastly, and critically, the additional final container sample for product release

testing alone would consume 39% of the gross product manufactured, significantly reducing world-wide product availability.

An example of the reduction of product availability caused by increased sampling and testing of final product is shown in Table 3. Even by minimizing testing requirements to preserve product for distribution, the number of vials of product required for release at 500 liter batch scale is still 29. Since only 75 vials of product are produced at such a scale, fully 39% of product would be lost by the testing requirements alone.

Product lyophilization would be a major rate-limiting step in production at small batch sizes as seen in Table 4. This table compares the cycle time and resources needed to produce 2250 vials from a single batch (15,000 liter scale) to the resources needed to produce the same 75 (gross) vials by 30 independent small runs. Actually, to keep up, 90 such small runs would need to be completed per week, not 30 as stated above. Clearly, energy resources required for such operation would be prohibitive. Additionally the complexity would raise concerns for its very practicality, even were all resources brought to bear. The complexity of such a process would also

require intensive maintenance programs to prevent on-line failures and would further likely reduce throughput and product availability.

The sheer number of repetitive processes which would need to be repeated and run with a high rate of quality assurance and GMP compliance if one assumed 90 batches per week are staggering and partially itemized on table 5. The increased difficulty of control and potential of failure at any point in such a complex process would add further risk to ongoing production. Production of 2250 vials in an ongoing process at small scale has a much higher risk of failure than a controlled process where 2250 vials are produced in a single batch.

If attempted, the staffing requirements to run production in such a mode are shown in table 6. At 500 liter scale, production would now take an estimated 736 operators running 90 independent batches per week with 18 filling stations per day and 90 lyophilization cycles to produce the same unit throughput as processing at 15,000 liter scale three times a week. The risk of human and machine error increases sharply at various points as shown in table 7.

The impact of operating at the two extreme scales (15,000 liters vs. 500 liters) may be best appreciated graphically by comparing Figure 1 vs. Figure 2. The increased number of runs represented in figure 2 is necessary to attempt to produce the same quantity of supply as in figure 1. Table 8 shows clearly how many more runs it takes to accomplish the same desired supply. Even if attempted, the final product availability would be decreased by the increased losses in handling smaller volumes (not considered in these calculations) and the increase in the percentage of product required for testing as demonstrated in table 3.

The ultimate impact of operating at small scale on product availability for medical use is shown in tables 9A and 9B. These tables calculates the quantity of product which would be produced by operating at three batch volume scenarios: 15,000 liters, 1500 liters, or 500 liters. Assumptions implicit in these calculations are: unit recovery during processing of 150 units per liter processed, rate limiting throughput by lyophilizer equipment such that only 5 batches could be processed per week, and 39% utilization of product in release testing at 500 liter batch scale as shown in table 2. From these calculations, it could be seen that operating at 500 liter batch size

would reduce product availability by 96%. Alternate scenarios might also be envisioned. For example, one could postulate increasing the number of batches which could be run to 10 per week by the purchase of additional equipment (tanks, lyophilizers, etc.) Although such an adjustment appears simple, the purchase of 5 more industrial scale lyophilizers could take as long as a year, followed by upwards of one year for installation, operational qualification, and validation. Plant expansions would additionally likely be required with their own time lines for operational qualification and validation. Even with such adjustments, one could calculate the impact in similar fashion to that of table 8 and find that even with such expansion, the availability of product would only be 23.79 million units per year or still 93.1% less product available than by operation at 15,000 liter batch size.

Tables 9A and B also show the impact that stabilizer albumin can have on the total donor number contained in a batch. By optimizing the stabilizer use, substantial improvement can be made in the number of donors in a batch even at the same process volume.

It is clear that continuing efforts should be made to improve the safety of all biological products which carry risk from infectious agents. These

improvements can be from advances in testing, from incorporation of better or yet to be discovered methods of inactivation, as well as from incorporation of separation methods to remove risk of co-purification of infectious materials.

The current generation of coagulation products in use represent such evolution in scientific discovery and technological advance. For one such product, a pasteurized plasma derived coagulation factor VIII purified to very high levels by immunoaffinity chromatography very high levels of virus removal have been demonstrated. Lipid enveloped viruses such as HIV have been shown to have a removal capacity of as much as 15 logs by a combination of pasteurization and purification. The same procedure has demonstrated 12 log removal capacity for EMC (a non-enveloped virus). It has become clearer in recent years that non-enveloped viruses can be significantly heterogeneous, however, such that viruses like parvovirus are difficult to remove with the same efficiency. For such agents additional methodologies will yet have to be incorporated.

Even as new methods are added or multiple existing methods chained together, validation of continued clinical efficacy and safety will be necessary to ensure that the stability of the therapeutic agent has not been

unknowingly compromised nor its structure altered to elicit antibody response.

As increasing knowledge is gained of the structure of the molecules with which we work, how to control their stability, and how to control their partitioning away from infectious agents, the potential of gaining additional safety increments by these methods is more promising than by operating at very small batch sizes, where generation of adequate supply and control of processing is generally inadequate.

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Table 1: Process Volume Relationship Vs. Batch Size

Scale / Fraction	15,000 L (3,000 L X 5)	1,500 L (300 L X 5)	500 L (100 L X 5)
Plasma	3,000 L	300 L	100 L
↓ Cryo	45 Kg.	4.5 Kg.	1.5 Kg.
↓ MoAb Column	100 L	10 L	3.3 L
↓ MoAb Eluate	150 L	15 L	5 L
↓ Freeze -Hold	7.5 L	0.75 L	0.25 L
↓ Combine Vol 5X	37.5 L	3.75 L	1.25 L
↓ AH Sepharose Column	4 L	0.4 L	0.133 L
↓ Eluate	3 L	0.3 L	0.1 L
↓ Final Sterile Bulk	9 L	0.9 L	0.3 L
↓ Gross Vials (1000 u/vl)	2250	225	75

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Table 2: Manufacturing Differences
Finishing Steps

	15,000 L Plasma Pool Final Lot	1,500 L Plasma Pool Final Lot	500 L Plasma Pool Final Lot
Monoclate-P Sterile Bulk	9000 mL	900 mL	300 mL
Vials Filled (1000 u/v)	2250	225	75
Lots per Week	3	30	90
Lots per Year	156	1560	4680
Lyophilizers Required	2	20	60
Fill Rooms Required	1	6	18
Plant Size Required	1X	6X	18X
Product Control Tests per Week	950	2300	6750
Product Release Tests per Week	63	630	1890
% of Total Consumed in Product Testing	2	18	39

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Table 3: Quality Control Sampling of Finished Vials

	Batch Size			
	15,000 L	5,000 L	1,500 L	500 L
• Sterility	20	20	20	8
• Bulk Sterility	5	5	5	5
• Appearance, Solution Time, pH, Total Protein, Identity, Hb, Iso's, and Murine	2	2	2	2
• Moisture, Azide, Calcium, Sodium, Mannitol and Histidine	3	3	3	3
• LAL, Pyrogens, and Safety	3	3	3	3
• Reserve Samples	8	8	8	8
Total Vials in Batch:	2250	750	225	75
Total Vials Used in Testing:	41	41	41	29
% of Lot Used in Testing:	2%	5.4%	18%	39%

Table 4:
Lyophilizer as Rate-Limiting Step

Capacity of 48 Sq.Ft. machine in 10 ml vials:	**3840 vials**	
Vials per 15,000 L batch:	2250	
Vials per 1,500 L batch:	225	
Vials per 500 L batch:	75	
Functional Operations of the lyophilizer per:	**2250 Vials**	**30x75 Vials**
1. Clean and Sanitize	3 Hours	90 Hours
2. Steam Sterilize	7 Hours	210 Hours
3. Freeze Product	13 Hours	390 Hours
4. One Drying Cycle	48 Hours	1,440 Hours
Full Cycle Time	71 Hours	2,130 Hours
Utilities Required:		
Cooling Water:	320,000 Liters	---
Electrical (Kw Hours):	2,130	63,900

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Table 5: Impact on Process, Plant Repetitive Operations

- Plasma Pooling
- Centrifugation
- Resuspension
- Filtration
- Precipitation
- Filtration
- Ultrafiltration
- Pasteurization
- Chromatography
- Sterile Filtration
- Washing of Vials
- Sterilization of Vials
- Filling
- Washing and Sterilizing of Filling Equipment
- Washing and Sterilization of Lyophilizer
- Lyophilization
- Facility sanitation
- Validations / Revalidation
- Documentations
- Quality Control Tests

Table 6: Staffing Requirements for Equal Throughput

	Batch Size		
	15,000 L	1,500 L*	500 L**
• Pooling, Cryo Purif:	33	50	198
• Chrom. Processing:	24	48	144
• Filling/Lyophilization:	12	72	216
• Sanitation:	10	30	50
• Quality Control Testing	16	64	128
• Personnel Total/process/week	95	*264	*736

* Assumes 30 Independent Batches per week with 6 filling operations per day and 30 Lyophilization cycles, to produce the same unit throughput

**Assumes 90 Independent Batches per week with 18 filling operations per day and 50 Lyophilization cycles, to produce the same unit throughput

Table 7: Risks Caused by Reduced Scale

- Increased personnel management
- Increased assays to monitor (LAL, Potency, Chemistries, Microbials.....etc...)
- Validation becomes excessive, limited.
- Paperwork, documentation, testing and review increases release time.

Figure 1: Flow Diagram 15,000 L Lot

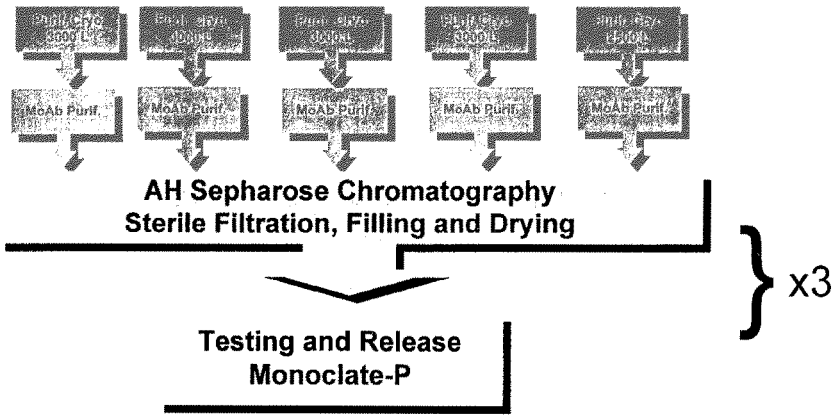

Figure 2: Flow Diagram 500 L Lots

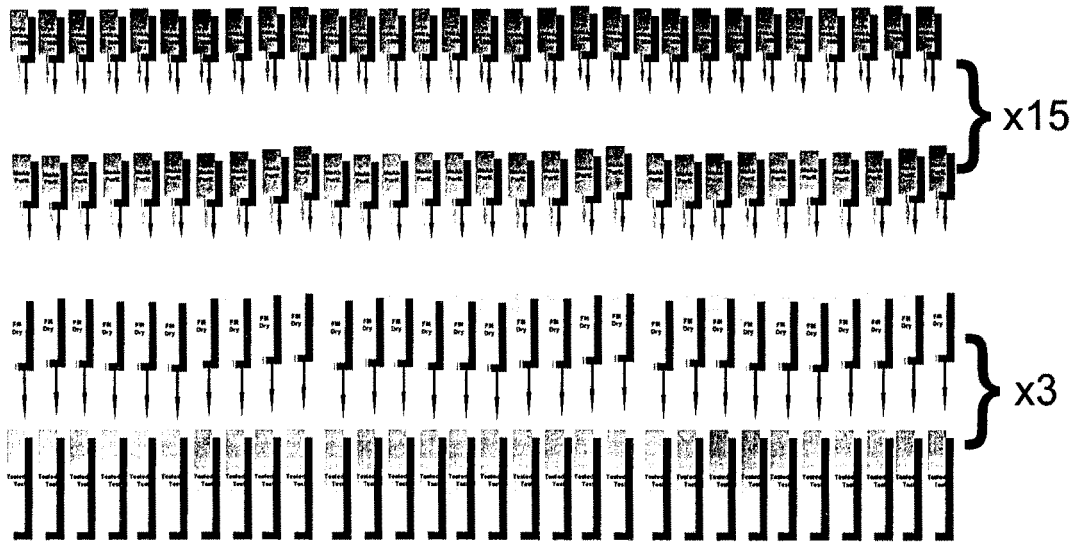

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Table 8: Number Of Operational Steps For Equivalent Weekly Throughput

Operation Step	15,000L (x3)	1500 L (x3)	500 L (x3)
Cryo	5 (15)	50 (150)	150 (450)
MAB Chrom	5 (15)	50 (150)	150 (450)
Fill & Dry	1 (3)	10 (30)	30 (90)
QC Test	1 (3)	10 (30)	30 (90)

Table 9: Batch Size vs. Product Availability
Monoclate-P (Factor VIII)

A:(Without Accounting For Albumin Stabilizer Donors)

Liters Per Batch:	15,000	5,000	1,500	500
Donors Per Batch Range:	11,600-17,500.	4,600-7,000.	1,100-1,800.	350-600.
Batches Per Week:	3	5	5	5
Vials Per Batch:	2250	750	225	75
Weeks Per Year:	52	52	52	52
% Net vials Released:	98	94.6	82	61
Potency (Units Per Vial:)	1,000	1,000	1,000	1,000
Million Units Produced Per Year:	344	185	48	12
Patients Served Per Year:	4,587	2,467	640	160

Table 9: Batch Size vs. Product Availability
Monoclate-P (Factor VIII)

B: (With Albumin Stabilizer Donors According To Current Practice)

Liters Per Batch:	15,000	5,000	1,500	500
Donors Per Batch Range:	53,000-81,000.	47,000-70,000.	43,000-65,000.	42,000-64,000.
Batches Per Week:	3	5	5	5
Vials Per Batch:	2250	750	225	75
Weeks Per Year:	52	52	52	52
% Net vials Released:	98	94.6	82	61
Potency (Units Per Vial):	1,000	1,000	1,000	1,000
Million Units Produced Per Year:	344	185	48	12
Patients Served Per Year:	4,587	2,467	640	160

Appendix 2

Pool Size Considerations In The Cohn Fractionation Process

Appendix 2

Pool Size Considerations In The Cohn Fractionation Process

The basic process for the manufacture of key plasma derivatives like human albumin and human gamma globulins was developed in the United States as a special government program headed by Dr. E.J. Cohn from Harvard University during World War II. The objective was to find a successful emergency treatment for blood loss in the battlefield to enable rapid casualty care. The Cohn fractionation process which resulted enabled the separation of plasma from whole human blood collected at centers and shipped to central manufacturing sites where critical life-saving fractions (derivatives or products) could be removed and utilized in the field (battlefield or civilian casualty conditions). The process as developed by Cohn utilized differential separation of proteins on the basis of specific controlled precipitation controlling the separation on a basis of protein charge and solubility manipulated by alcohol concentration, ionic strength of the solution ("salt concentration"), protein concentration, and temperature. The separation method allowed the ready division of plasma into discrete separated fractions where the therapeutic proteins could be removed and put into

pharmaceutically accepted formulations. The first therapeutic applications were for the human gamma globulin and albumin fractions which could be provided in high purity in different fractions, respectively Fraction II and Fraction V. The separation steps needed were many and complex, but provided the opportunity for separating many of the proteins which could be found in plasma into different fractions for clinical research or application. The Cohn Fractionation Method (Figure 1) is still in place today among virtually all fractionators, but variations of methodology and technology exist between them, and manufacturers' have additionally learned how to separate other therapeutic proteins (such as Factor VIII for treating hemophilia A from cryoprecipitate and Factor IX for treating hemophilia B from the cryoprecipitate poor plasma (CPP), as well as other therapeutic proteins).

Plasma proteins are present in the starting plasma at different concentrations. Some are present at relatively higher levels but require high doses for treatment (gram quantities like albumin or IVIG). Others are present at very trace levels in plasma and require high purification and concentration from large quantities of starting plasma to enable sufficient recovery for treating large numbers of patients. Since significant losses occur in separation and

fractionation methods (generally the more processing steps, the more losses), large volume methods are generally needed to generate sufficient product for patient treatment.

As explained in Appendix 1 for operational choices for the preparation of coagulation Factor VIII, similar impacts operate on selecting pool sizes for the manufacture of products from the Cohn fractionation method (Human Albumin, Human IgG (IVIG), Alpha-1-Antitrypsin, and others). Each process has to be controlled according to strict GMP requirements, as well as scientific ones. Samples are removed throughout the process for testing and assurance that the process is behaving as expected. Unlike the preparation of chemical therapeutic molecules from organic synthesis, the preparation and purification of biological therapeutics is subject to greater variation. Biological systems vary, whether from individual variances among plasma donors or whether from harvests from cell culture systems, and the separations of large protein molecules have some intrinsic variation. These must be carefully monitored and controlled for and represent some losses during processing, the smaller the fractionation stream, obviously the larger the percentage of losses. The greater losses, however, can occur during final

product quality control testing (or even during extensive and ongoing process validation studies). In those cases, small fractionation pools generate small levels of product and sampling requirements constitute a greater percentage loss of product.

The final consideration to be discussed here relating to products obtained from the Cohn fractionation method or its variations relates to process scale and process equipment. The scale of operations for these methods is large (for reasons explained above), the resulting equipment (tanks, pumps, ultrafilters, filters, freeze-driers) is hence also large, and the plant, therefore, is also large and complex. The existing size of the plants is a critical parameter, not only because it represents the resource available today, but also because substantially decreasing process volumes immediately raises questions and concerns of processing equivalence and impacts on product safety and efficacy. Simple examples of such impacts are apparent: if the volume to go into a large tank is decreased by half (or more) and the process is dependent on precipitation and separation phenomena (as the Cohn process is), the decreased volume may no longer fit with the stirring system in place and result in foaming and denaturation if the stirrer now "whips" during an

alcohol addition and separation step. Even if the impact isn't as dramatic, altered tank geometries and alcohol additions and stirring modalities can change the nature of the precipitant, the nature of the impurities which carry forward, and the stability of the product which is derived. The more dramatic the changes, the greater the need for process validation and supporting documentation (pharmacology/toxicology studies; stability studies; perhaps even clinical data on pharmacokinetics or immunogenicity). That such documentation is needed is ever increasingly apparent through current GMP and Quality Assurance systems thinking as formulated by the FDA and consistent with good science and technology. If the end result is a highly desired one, changes can occur. The greater the scope of the change, however, the greater the impact on the operation. Generally, we can assume that to make even one large tank change in such a plant, that walls would have to come down, environmental control systems would be impacted (air and water system shutdowns and revalidation), installation and qualification of new tanks and controls, and process qualification of the completed system even unto making new "conformance lots" demonstrating return to controlled conditions. If multiple changes were instituted in fractionation plants, the potential for simultaneous large scale shutdown of multiple facilities could be

envisioned with resulting interruptions in supply. Especially with lead times of large scale equipment of as much as a year or more, with extensive renovation operations running probably at least a year, and with revalidation operations taking often two years, a time period of change of four years could easily be envisioned, during which time normal production could not be done in that facility. Alternatively, new plants with small scale operations could be entertained. However, as shown for the Factor VIII case (Appendix 1), the complexity of operation increases substantially, even while the supply from one unit operation drops, the efficiency decays, and costs increase.

It is incumbent upon manufacturers of critical drugs not only to continue to supply product reliably, but to look for improvement opportunity - improvements in furthering safety and efficacy, improvements in technology to improve supply, improvements in technology and technique to gain better process control and quality assurance, and, we have understood, improvements to patients and payors with regard to cost-benefit.

Although we believe that substantial changes of process volumes are difficult to achieve, potentially disruptive of supply, and even in instances frought

with risk of reduced safety, we have asked where there are opportunities within the existing fractionation system where improvements in control and donor exposure can be implemented without creating significant regulatory or supply difficulties and where risk reduction benefit could result, at least to the patient infrequently exposed to pooled plasma derivatives. (In discussing risk reduction in relationship to pool sizes, some confusion can exist between the relationship of process volume as expressed in liter quantities, the total donations contained in a batch, and the number of donors contained. To facilitate further discussion, definitions for these terms are supplied in Table 1.)

We believe that we have identified opportunities for Centeon to reduce the total donor exposure from a given batch and have initiated the following program of improvement (Table 2):

1. Identify where the range of production batch sizes (total donor exposure) is large. Identify outliers in each product category and contributors to this condition and reduce the range of variation, diminishing the top end of the range to the greatest extent.

2. Eliminate the practice of recovery of units such as "packaging defects" and other similar categories of product vials where the product quality is believed intact, but where minor imperfections still qualified recovery by addition of sporadic units to another lot in process.

   Although this practice conserves the supply of product from valuable national resources, it can contribute disproportionately to increasing donor exposure from any given batch. We have discontinued this practice for all new production since January 1997.

3. Reduce the combined sublots (subsections) in processing Factor VIII.
4. Limit the addition of stabilizer human albumin in the use of manufacture of a therapeutic product such as Monoclate-P or Gammar P I.V. to ensure that not more than one lot of albumin is used in the manufacture of a specific lot of final product.
5. To reduce the donor increase contributed by the human albumin stabilizer, prepare the stabilizer albumin from a special batch of albumin.
6. Reduce the total pool from which sections are pulled for manufacture of Mononine (Factor IX).

7. Limit the number of donors going into the Cohn process by ensuring that the donor exposure will result from not more than two plasma pool equivalents being utilized for construction of the CPP which will start fractionation, rather than three.
8. In the preparation of Gammar P I.V., limit the donor exposure which will result from combination of Fraction II+III pastes so that donors from not more than two such equivalent pastes are combined, rather than three.
9. Control the plasma collection and distribution system to enable 50% repeat plasma donors going into a pool to reduce the overall exposure rate from donors, even in equivalent batch sizes.

We believe that by implementation of these intiatives, that Centeon can decrease the variation of donor numbers which occurs in processing and reduce the overall maximum number of donors associated with any given batch of product. Specific product by product changes which we believe can generate this as well as equipment related opportunities which may offer other opportunities for improvement will be reviewed with FDA to ensure compliance with cGMP and control of quality assurance before process changes are intiated.

Appendix 2 Report

Figure 1: Centeon Production of Fractions and Products

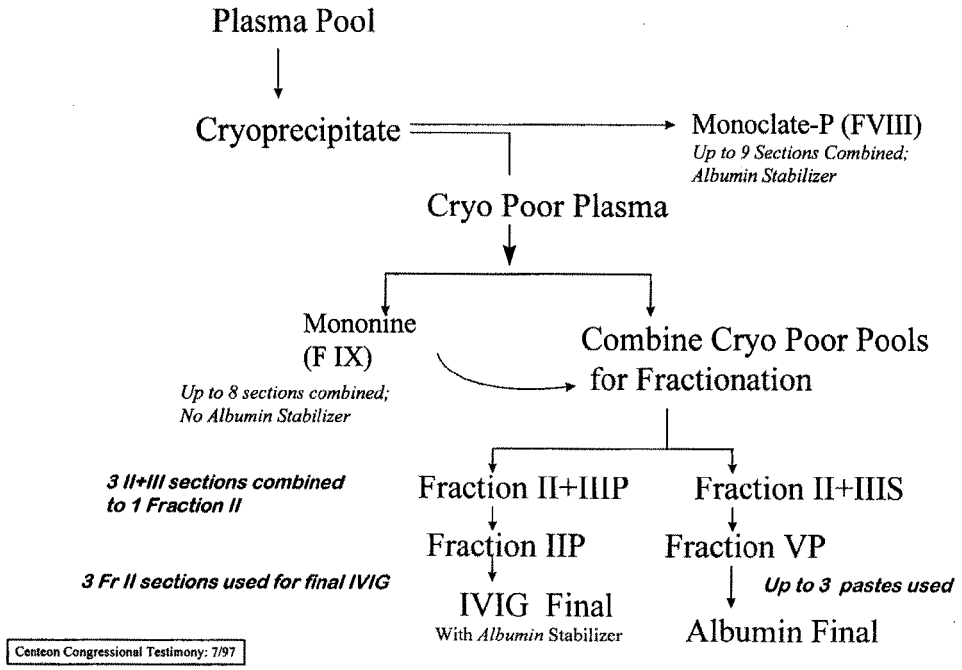

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Table 1: Definitions Involving Pool Size

- Total Liters: Total Equivalent Volume Contributed By Combining Sections or Sub-batches.
- Total Donations:
  - For Plasmapheresed Plasma = Liters Volume/0.8 Liters Per Donation
  - For Recovered Plasma = Liters Volume/0.25 Liters Per Donation
- Total Donors:
  - Donations Divided By Donor Repeat Factor
  - Examples: Divide by 1.0 If Donors Don't Repeat
  - Examples: Divide by 0.834 If 50% Repeat Donors

Table 2: Donor Pool Improvement Program

1 Identify And Decrease The Total Donor Exposure Range

2 Eliminate Add-back Of Recovered Units

3 Reduce Combined Sections On Factor VIII

4 Limit Addition Of Stabilizers To A Single Lot At A Time

5 Make A Special Small Lot Of Stabilizer For Use In Factor VIII

6 Reduce The Pool For Beginning Factor IX Production

7 Limit The Cryo Poor Pool For Cohn Process To Two Sections

8 Combine Only Two Fr II+III Pastes For IVIG

9 Control Repeat Donor Rate To Gain 50% Repeat Donors In Pool

Appendix 3

Centeon Research Activities: Safety Initiatives

Appendix 3

Centeon Research Initiatives To Further Safety

To gain better knowledge of where threats might originate to safety of products derived from plasma, a variety of safety initiatives have been undertaken. To provide increased surveillance opportunities and gain expertise in a broad range of scientific disciplines, Centeon has assembled a scientific counsel of eminent specialists. The Transmissible Agents Scientific Council (named TASC), constituted in 1996, has met between one and two times yearly and fulfills the safety objectives stated in its mission in Figure 1. Expertise represented on TASC encompasses a comprehensive range of infectious organisms, with agents causing prion diseases represented by specialists from four countries (Figure 2). Specialists in epidemiology and emerging infectious agents are also represented and add to the breadth of the group. To ensure an appropriate awareness of the potentials and/or the limitations of fractionation to the group, training seminars in fractionation initiated the early meetings. To ensure a high level of commitment to the counsel, Centeon membership includes the Vice-President of Clinical Research and Development, the Vice-President of Pre-Clinical Research and Development, the Head of Virology Research, and company specialists in validation and process development. A scientist with long-term research expertise in prion science and CJD has been added to Centeon and also sits on TASC.

Although the scientific and medical communities have been unable to agree on the specific risk which the CJD agent represents to plasma products and have termed it a "theoretical risk," Centeon has invested significant resources in furthering research to understand the agent and developing methods to improve its detection and elimination. Figure 3 provides details on a research contract initiated in 1996 with the laboratory of Dr. Stanley Prusiner, the world expert in prion science, to enhance understanding of the agent and develop a specific rapid bioassay in transgenic animals sensitive to human prions. Specific goals have been agreed upon and research activities initiated in 1996 (Figure 4). As part of the program, conferences have been held exchanging specific knowledge between the laboratories (Centeon's and Prusiner's) to promote the

opportunity for each of the laboratories to better understand how their particular and unique knowledge could be applied by the other (Figure 5). Specific areas under discussion are shown in Figure 6 and interim progress will be discussed with FDA as data become available.

Figure 1

Transmissible Agents Scientific Council (TASC)

- Mission
  - Council on Safety Aspects of Plasma Derivatives
  - Guidance on New Biological Product Assessments
  - Consideration of New Transmissible Agents
  - Recommendations on New Tests for Improving Product Safety

Figure 2

Transmissible Agents Scientific Council (TASC)

Expertise	Country
CJD	U.S./France
Prions	U.S.
BSE/Scrapie	Scotland
Viruses;Cell Culture;Viral Safety	France
Hepatitis Viruses; Virology	U.S.
HIV; Epidemiology; Virology	Germany
Epidemiology; Emerging Agents	U.S.

Figure 3

Centeon R&D: CJD Initiatives

- Research Contract With Laboratory of Dr. Stanley Prusiner Initiated 1996
  - Aims of Our Collaboration
    - » To Establish A Rapid and Sensitive BioAssay for Human PRP^{SC}
    - » To Gain Access To Test Systems To Explore Removal Methods for Prions
    - » To Extend the Bioassay to Bovine and Ovine Prions
    - » To Ultimately Develop a Cell-Free Assay System

Figure 4

Centeon R&D: CJD Initiatives

- Evaluate Opportunities With Prusiner Laboratory For Moving Validation Options Forward
  - Agreed:
    - Establish and Characterize Human Prion Standard Inoculum
    - Consider Approaches To Spiking/Elimination Experiments On Human Plasma

Figure 5

Research Dialogue: Prion Knowledge/Plasma Fractionation

- Gain Detailed Knowledge of Prion Structure/Chemistry Which Could Be Applied In Detection/Removal Methods With Plasma Proteins
- Provide Information on Human Plasma Fractionation and Methods Used To Increase Safety

Figure 6

Detection/Assay/ Validation Potentials

- How to assess risk of transmission from cellular components or plasma derivatives
- Need for titered human inoculum
  - Characterized molecular structure
  - Characterized size/aggregation state/solubility
- How to conduct spiking and removal experiments?
  - Use of scrapie or human agent?
  - Differences in structure/separation artifacts?
- Testing end-stage CJD patients for circulating agent
  - Source material
  - Cellular components
  - Tracking PRP^{sc} in specific fractionation schemes

Mr. SHAYS. Ms. Preston, and then we'll get to questions.

I would like to note for the record that we're joined by the truly distinguished chairman of our committee, Mr. Burton, and we appreciate his being here. I was thinking as he walked in he thought, what am I getting myself into here, bringing out one chart after another. This is what one of your committees does, Mr. Chairman.

Ms. PRESTON. Mr. Chairman, and members of the committee, ladies and gentlemen, I am Sue Preston, vice president of Quality and Regulatory Affairs, Alpha Therapeutic Corp., located in Los Angeles, CA. We process human plasma into life-saving treatments for patients with immunodeficiency, hemophilia, and trauma victims. Immunoglobulins, Factor VIII, Factor IX, and albumin are some of the products that are licensed by the Food and Drug Administration. We have additional biologic products and drugs in clinical trials. Alpha Therapeutic Corp. markets products in the United States and in over 50 countries worldwide.

I would like to talk to you today about the multiple measures that our industry and Alpha Therapeutic Corp. have incorporated to ensure safe and effective products. Reduction in the risk of donor exposure, sometimes referred to as pool size reduction, is only one measure of many measures, some of which are more than effective and have a broader impact. The chart depicts many of the voluntary and mandatory safety measures incorporated in our processes.

Beginning with the population of donors, we voluntarily have implemented industry quality plasma program standards for viral marker testing. Each and every donor from whom we collect plasma, we have an extensive medical screening and testing program. The FDA has regulations which require asking the donor about high-risk behavior, medical history, and CJD. We have voluntarily added several additional requirements, such as an age limit less than 60 years, and deferring donors with a history of corneal transplants to further preclude the risk of CJD. Every donor is screened against the National Donor Deferral Registry for a history of positive viral marker testing. Furthermore, we conduct drug screening as part of our donor acceptance program.

Every donor is examined at each donation for health status, and samples of blood are tested for normal levels of protein and hemoglobin as required by regulations. Alpha Therapeutic Corp. performs physician-supervised extended medical screening and adheres to the industry voluntary standards for accepting donations from only qualified donors; that is, those with two or more donations with all negative viral marker tests.

Over 95 percent of our donations come from repeat donors. These donors are well known to our plasmapheresis medical staff, as we see these donors several times each month. Our donors in plasmapheresis centers are part of the communities in which they're located.

The next step in the process is testing samples from each and every donation for the presence of viral markers, such as hepatitis B antigen, hepatitis C antibodies, human immunodeficiency virus antigen, and antibodies.

At our Memphis laboratory, we test for the level of liver enzymes so that donors with liver disease are deferred appropriately. We

have a double identification system on our plasma collection bottles and our sample tubes so that sample mixups with test results are virtually eliminated. We adhere to the industry standards with respect to holding plasma units to assist in retrieving units from donors who subsequently test positive for viral markers. We maintain backup samples of our donations if additional testing is necessary.

Alpha Therapeutic Corp. has implemented many voluntary measures such as testing for viral markers in minipools over and above the individual units testing. For instance, we utilize a different test kit for detection of HIV antibodies to exclude test errors. We have begun clinical trials on the ability of the most sensitive test method available, polymerase chain reaction, in minipools to detect viral nucleic acid material from HIV and HCV.

Our manufacturing process begins with the voluntary testing of samples from our plasma pool after all of the donations are pooled for hepatitis B antigen and hepatitis C antibodies and with two different test kits for the absence of HIV antibodies.

We have already incorporated steps to reduce the donor exposure in the final products as outlined in our IPPIA presentation.

For our products, safety is a combination of many factors, but the most important for currently known pathogenic acts and possibly for those that are unknown remains our manufacturing process itself.

The FDA mandates the viral inactivation step of heat treatment or pasteurization for albumin products. In each of our processes, we incorporate steps to remove or inactivate viruses such as solvent detergent treatment that inactivates HIV, HBV, and HCV very efficiently.

Other steps have been added to reduce the potential for other types of viruses such as hepatitis A or parvovirus. We are exploring a step with some preliminary information on CJD infectivity removal. However, much additional research will be necessary to confirm these very preliminary results.

During the course of the manufacturing process, samples are taken for testing. Samples of the final container batch are subjected to a large battery of tests. Sterility, potency, purity, safety, and stability are mandated by the regulations or in our product licenses. We have implemented voluntary testing for hepatitis B antigen and antibodies for hepatitis C and HIV.

Since March 1996, Alpha Therapeutic Corp. has also tested samples from each final container batch for the absence of viral nucleic acids by polymerase chain reaction, for hepatitis A, hepatitis B, hepatitis C, and HIV. Only negative lots are released for distribution.

Alpha Therapeutic continues to monitor the product safety once it leaves our doors through marketing surveillance. We report adverse events promptly to the FDA. We conduct ongoing clinical trials with our products to continuously monitor the safety and efficacy as we improve processes. We seek and receive constant feedback from recipients of our products. In the event that we have discovered subsequent to product distribution a potential risk, we work with our customers, consumer groups, and regulatory agencies to take the appropriate actions to eliminate the risk through

quarantine or recall notifications. We support the initiatives as described in the IPPIA testimony with respect to notification.

We will never rest in our vigilance for safety. Our scientists work tirelessly to develop improved methods for ensuring safety through better manufacturing processes or improved tests. We cooperate with other corporations to develop more sensitive methods for detecting disease. We participate in the research consortium for plasma science for developing better viral inactivation processes. We encourage our industry organization to increase standards of excellence. We continue to work with regulatory agencies around the world——

Mr. SHAYS. OK.

Ms. PRESTON [continuing]. To ensure we can supply the most safest and efficacious products. Thank you.

Mr. SHAYS. Thank you.

[The prepared statement of Ms. Preston follows:]

Foreword

This document is in response to an invitation from the Congress of the United States House of Representatives' Committee on Government Reform and Oversight, Subcommittee on Human Resources and Chairman Christopher Shays for Ms. M. Sue Preston's testimony, on behalf of the Alpha Therapeutic Corporation, for a scheduled hearing on July 31, 1997, concerning the safety implications of plasma pool sizes in the manufacture of fractionated plasma products.

The information submitted herein contains trade secrets and/or is confidential and may not be revealed or disclosed without the prior written authorization of the Alpha Therapeutic Corporation.

The lifesaving benefits of plasma have been recognized throughout history. This liquid portion of human blood contains essential components including blood clotting factors, immune globulin antibodies and plasma volume expanders. It was in the 1940s, when researchers at Harvard University launched a project to "fractionate" plasma, or separate its components, that the modern plasma age was born.

From the very beginning, Alpha Therapeutic Corporation has provided leadership in sourcing, manufacturing and distributing plasma derived pharmaceutical products. Alpha evolved from Courtland Laboratories, which started in Los Angeles in 1948 and later became Abbott Laboratories. Alpha was incorporated in California in 1978 as a wholly owned subsidiary of the Green Cross Corporation of Osaka, Japan.

With continued growth, Alpha now has worldwide sales nearing $400 million and employs over 2,600 dedicated people, who work in our manufacturing facilities, research laboratories, warehouses, donor centers and corporate offices. Other facilities include international subsidiaries, the Memphis testing laboratory, a specialty plastics plant and Alpha Therapeutic Services, a subsidiary which provides home infusion products and services.

This involvement in the entire plasma production process reflects Alpha's commitment to people, from the employees who make our products to the patients who receive them.

alpha®
THERAPEUTIC CORPORATION

ALPHA PRODUCTS

Albutein ®	- Albumin (Human)
Plasmatein ®	- Plasma Protein Fraction
Venoglobulin ®	- S-Immune Globulin Intravenous (Human) Solvent Detergent Treated, Liquid
AlphaNine ®	- Coagulation Factor IX (Human) Affinity Purified Solvent Detergent Treated and Viral Filtered
Profilnine ®	- Factor IX Complex Solvent Detergent Treated
Alphanate ™	- Antihemophilic Factor (Human), Solvent Detergent and Heat Treated
Profilate ®	- Antihemophilic Factor (Human), Solvent Detergent Treated
Source Plasma	- Collected at primarily Alpha owned donor centers
Plasma Fractions	- Cryoprecipitate, Fraction II + III and Fraction IV
Softgoods	- Disposable tubing and containers used in the plasmapheresis procedure
Testing Services	- Full panels for both blood and plasma specimens

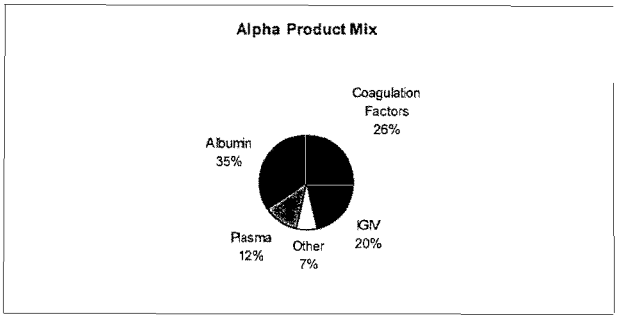


Table of Contents

I. INTRODUCTION

It is the understanding of Alpha Therapeutic Corporation that the Committee on Government Oversight and Reform, the Subcommittee on Human Resources and its chairman Christopher Shays have scheduled this hearing in an effort to discuss arguments in favor of limiting plasma pool sizes in an effort to reduce the risk of infectious disease transmission and to reduce the impact of product recalls. A copy of the letter of invitation to testify is provided following this introduction.

This testimony is intended to discuss the safety implications of plasma pool sizes in Alpha Therapeutic Corporation's fractionated plasma products. This testimony considers the risks and safety associated with Alpha Therapeutic Corporation plasma products and addresses the efforts to detect and remove the agent of Creutzfeldt-Jacob Disease (CJD). Furthermore, we provide testimony on considerations for recall and notification efficiency.

As we discuss our therapeutic products, we must also consider the number of other products that incorporate our products as reagents and stabilizers. A number of recombinant DNA products utilize our albumin products during the course of manufacture or as a final container stabilizer. Some contrast agents are manufactured with human albumin as a base. Furthermore, as the need for tissue preservation, such as for *in vitro* fertilization, increases, our products touch more recipients.

Congress of the United States

House of Representatives

SUBCOMMITTEE ON HUMAN RESOURCES
Christopher Shays, Connecticut
Chairman
Room B-372 Rayburn Building
Washington, D.C. 20515
Tel: 202 225-2548
Fax: 202 225-2382
E-mail: hr.groc@mail.house.gov

July 17, 1997

Ms. M. Sue Preston
Vice President
Quality & Regulatory Affairs
Alpha Therapeutic Corporation
2410 Lillyvale Avenue
Los Angeles, CA 90032

Dear Ms. Preston:

Our subcommittee, with oversight responsibilities for U.S. Department of Health and Human Services' (HHS) agencies, has scheduled a hearing for July 31, 1997 on the safety implications of plasma pool sizes in the manufacture of fractionated blood products. The subcommittee would benefit from hearing your views and we invite you to testify.

The purpose of the hearing is to review the risks associated with pooled plasma products. We will consider: the effectiveness of surveillance efforts to detect the presence of Creutzfeldt-Jakob Disease (CJD) in the blood supply, new research on the possible transmission of CJD through the blood supply, and the relationship between plasma pool size and the risk of infectious disease transmission and product recall efficiency. Please focus your testimony on these issues and your recommendations for addressing these questions.

The hearing will be held in Room 2247 of the Rayburn Building at 10:00 a.m. Please summarize your written testimony in five minutes so that we will have maximum time for questions and discussion.

Witnesses are asked to provide 100 copies of their prepared testimony (with 20 of these unstapled), delivered to the subcommittee office, Rayburn B-372, at least 48 hours prior to the hearing. In order to facilitate printing of the hearing record, we would also appreciate receiving an exact copy of your testimony on a 3.5 inch computer diskette. (See enclosed form.)

Pursuant to House rules, please submit with your testimony a *curriculum vitae* and a written disclosure of the amount and source (by agency and program) of any federal grant (or subgrant) or contract (or subcontract) received during the current fiscal year or either of the two previous fiscal years by you or the organization or entity you represent.

Under the Congressional Accountability Act, the House of Representatives must be in compliance with the Americans with Disabilities Act. Persons requiring special accommodations should contact Jared Carpenter of the subcommittee staff at least 4 business days prior to the scheduled hearing.

If you have any questions, please call Anne Marie Finley of the subcommittee staff at (202) 225-2548.

We look forward to your testimony at the hearing.

Sincerely,

Christopher Shays
Chairman

cc: Rep. Dan Burton
Rep. Vince Snowbarger
Rep. Edolphus Towns
Rep. Henry Waxman

II. SUMMARY

A. Oral Testimony by M. Sue Preston

Good Afternoon Ladies and Gentlemen. I am Sue Preston, Vice President of Quality and Regulatory from Alpha Therapeutic Corporation located in Los Angeles California. We process human plasma into life-saving treatments for patients with immunodeficiency, hemophilia, and trauma victims. Immunoglobulin, Factor VIII, Factor IX, and albumin are some of the products that are licensed by the Food and Drug Administration. We have additional biological products and drugs in clinical trials. Alpha Therapeutic Corporation markets products in the United States and in over 50 countries in the world in compliance with US and international regulatory requirements.

I would like to talk with you today about the multiple measures that our industry and Alpha Therapeutic Corporation have incorporated to assure safe and effective products. Reduction in the risk of donor exposure, sometimes referred to as pool size reduction, is only one measure of many alternative measures that are much more effective and have a broader impact. This chart depicts many of the voluntary and mandatory safety measures incorporated in our process. Beginning with the population of donors, we voluntarily have implemented industry Quality Plasma Program standards for viral marker testing.

For each and every donor from whom we collect plasma, we have an extensive medical screening and testing program. The FDA has regulations which require asking the donor about high risk behavior, medical history and CJD risk. We have voluntarily added several additional requirements such as an age limit less than 60 years and to defer donors with a history of corneal transplants to further preclude the risk for CJD. Every donor is screened against the National Donor Deferral Registry for history of positive viral marker testing. Furthermore, we conduct drug screening as part of our donor acceptance program. Every donor is examined at each donation for health status and samples of blood are tested for normal levels of protein and hemoglobin as required by the regulations. Alpha Therapeutic Corporation performs physician-supervised extended medical screening and adheres to the industry voluntary standards for accepting donations from only qualified donors, those with two or more donations with all negative viral marker tests. Over 95% of our donations come from repeat donors; these donors are well known to our plasmapheresis center medical staff as we see these donors several times each month. Our donors and plasmapheresis centers are an integral part of the communities in which they are located.

The next step in the process is testing samples from each and every donation for the presence of viral markers such as hepatitis B antigen, hepatitis C antibodies, human immunodeficiency virus (HIV) antigen and antibodies as required by the FDA. At our Memphis Laboratory, we test for the level of liver enzymes so that donors with liver disease are deferred appropriately. We have a double identification system on our plasma collection bottles and our sample tubes so that sample mix-ups are virtually eliminated. We adhere to the industry standards with respect to holding plasma units to assist in retrieving units from donors who subsequently test positive for viral markers. We maintain back up samples of our donations as a precaution if additional testing is necessary.

Alpha Therapeutic Corporation has implemented many voluntary measures such as testing for viral markers in mini-pools over and above the individual unit testing. For instance, we utilize a different test kit for detection of HIV antibodies to also exclude test errors. We have begun clinical trials on the ability of the most sensitive test available, polymerase chain reaction in mini-pools to detect viral nucleic acid material from HIV or HCV.

Our manufacturing process begins with voluntary testing of samples from the plasma pool for hepatitis B antigen and hepatitis C antibodies and with two different test kits for the absence of HIV antibodies. We have already incorporated steps to reduce the donor exposure in the final products as outlined in the IPPIA presentation.

For our products, safety is a combination of many factors, but the most important for currently known pathogenic agents and, possibly those that are unknown, remains the manufacturing process itself. The FDA mandates the viral inactivation step of heat treatment or pasteurization for albumin products. In each of our processes, we incorporate steps to remove or inactivate viruses such as solvent-detergent treatment that inactivates HIV, HBV and HCV very efficiently. Other steps have been added to reduce the potential for other types of viruses such as hepatitis A or parvovirus. We are exploring a step with some preliminary information on CJD infectivity removal. Additional research efforts will be necessary to confirm the preliminary results.

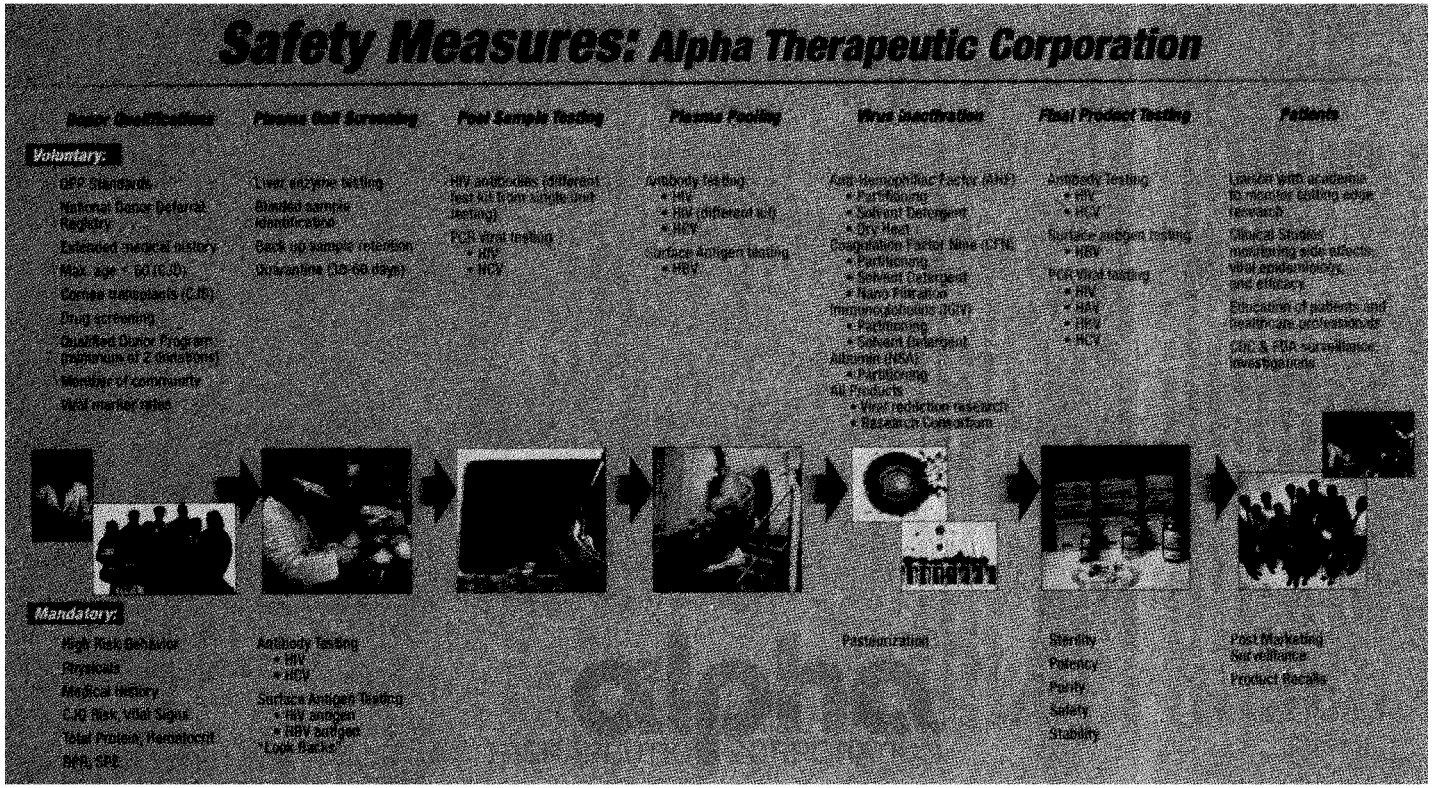
During the course of the manufacturing process, samples are taken for testing. Samples of the final container batch are subjected to a battery of tests. Sterility, potency, purity, safety and stability are mandated by the regulations or in our product licenses issued by the FDA. We have implemented voluntary testing for hepatitis B antigen and antibodies for hepatitis C and HIV. Since March 1996, Alpha Therapeutic Corporation has also tested samples from each final container batch for the absence of viral

nucleic acid by polymerase chain reaction for hepatitis A, hepatitis B, hepatitis C and HIV. Only negative lots are released for distribution.

Alpha Therapeutic Corporation continues to monitor the product safety once it leaves our doors through marketing surveillance. We report adverse events promptly to the FDA. Furthermore, we conduct on-going clinical trials with our products to continuously monitor the safety and efficacy as we improve our processes. We seek and receive constant feedback from recipients of our products. In the event that we have discovered subsequent to product distribution, a potential risk, we work with our customers, consumer groups and regulatory agencies to take the appropriate actions to eliminate the risk though quarantine or recall notifications. We support the initiatives as described in the IPPIA testimony.

We will never rest in our vigilance for safety. Our scientists work tirelessly to develop improved methods for ensuring safety through better manufacturing processes or improved tests. We cooperate with other corporations to develop more sensitive methods for detecting disease. We participate in the research consortium for developing better viral inactivation processes. We encourage our industry organizations to embrace standards of excellence. We continue to work with regulatory agencies around the world to assure that we supply the safest and most efficacious products through implementation of proactive safeguards and improved processes. Alpha Therapeutic Corporation will never rest in our vigilance for safety.

Thank you for your attention to our presentation.


Safety Measures: Alpha Therapeutic Corporation
Donor Qualifications
Plasma Unit Screening
Post Sample Testing
Plasma Pooling
Virus Inactivation
Final Product Testing
Patients
Voluntary:
GPP Standards
National Donor Deferral Registry
Extended medical history
Max. age = 60 (65 D)
Cornea transplants (CJD)
Drug screening
Qualified Donor Program (minimum of 2 donations)
Member of community
Viral marker retest
Liver enzyme testing
Blended sample identification
Back up sample retention
Quarantine (10-60 days)
HIV antibodies (different test kit from single unit testing)
PCR viral testing
• HIV
• HCV
Antibody testing
• HIV
• HIV (different kit)
• HCV
Surface Antigen testing
• HBV
Anti-Hemophilic Factor (AHF)
• Partitioning
• Solvent Detergent
• Dry Heat
Coagulation Factor Nine (CFN)
• Partitioning
• Solvent Detergent
• Nano Filtration
Immunoglobulins (IGIV)
• Partitioning
• Solvent Detergent
Albumin (HSA)
• Partitioning
All Products
• Viral reduction research
• Bacteria in Core Room
Antibody Testing
• HIV
• HCV
Surface antigen testing
• HBV
PCR Viral testing
• HIV
• HAV
• HCV
• HCV
Lawsuits with enrollment to monitor adding value research
Clinical Studies monitoring safety, viral contaminants, and efficacy
Education of patients and healthcare professionals
CDC & FDA surveillance investigations
Mandatory:
High Risk Behavior
Physicals
Medical history
C.IQ Risk, Vital Signs
Total Protein, Hematocrit
BPR, SPE
Antibody Testing
• HIV
• HCV
Surface Antigen Testing
• HBV antigen
• HBV antigen
Look Backs
Pasteurization
Sterility
Potency
Purity
Safety
Stability
Post Marketing Surveillance
Product Recalls

II. C. Curriculum Vitae, M. Sue Preston

Title: Vice President, Quality & Regulatory Affairs

Business Address:

Alpha Therapeutic Corporation
5555 Valley Boulevard
Los Angeles, CA 90032
213-227-7580
Facsimile: 213-227-9053

Education:

1981 - 1985	University of Maryland, College Park Graduate Coursework, Biochemistry and Physiology
1974	Lycoming College, Williamsport, PA BA, Cum Laude, Biology Major, Chemistry Minor

Professional Experience:

1997 to Present	Vice President, Quality and Regulatory Affairs Alpha Therapeutic Corporation Los Angeles, CA 90032
1993 -1997	Vice President, Regulatory Affairs Alpha Therapeutic Corporation Los Angeles, CA 90032
1992 - 1993	Director, Regulatory Affairs and Quality Assurance Medarex, Inc. 12 Commerce Avenue West Lebanon, NH 03784
1990 - 1992	Director Regulatory Affairs and Quality Assurance Trancel Corporation 1201 Wakeham Avenue Santa Ana, CA
1988 - 1990	Manager, Regulatory Affairs Baxter Healthcare Corporation 550 North Brand Avenue Glendale, CA

1980 - 1988	Biologist and Regulatory Coordinator Plasma Proteins Laboratory FDA/CBER, Division of Blood and Blood Products Bethesda, Maryland
1974-1980	Biologist NIH Bethesda, MD

Professional Organizations:

Drug Information Association
American Heart Association
Tissue Culture Association
Regulatory Affairs Professional Society
American Society of Quality Control

Honors and Awards

Commissioners Special Citation - 1988
Quality Performance Award - 1985
Commendable Services Award - Public Health Service Division - 1984

Presentations

June 1997	Practical Implications of Pool Size Reduction American Blood Resource Association Plasma Forum Washington, DC
December 1996	Plasma Product Safety & Supply Blood Products Advisory Committee Rockville, Maryland
November 1989	International Regulatory Strategy for Recombinant Antihemophilic Factor Baxter International Brussels, Belgium
January 1986	Inactivation of AIDS Virus by Ethanol Fractionation Workshop on the Safety of Intravenous Immunoglobulin Preparations with Respect to Transmission f Hepatitis and AIDS Viruses Central Laboratory Blood Transfusion Service Swiss Red Cross Berne, Switzerland
September 1982	High Performance Chromatography Profiles of Plasma Derivatives Workshop on the Molecular Integrity of Plasma and Cell-Derived Products FDA, OB Bethesda, MD
May 1980	A Probe for the Organization of Beta-Ardenergic Receptor Regulated Adenylate Cyclase System in Turkey Ethrocyte Membranes by Use of a Complementation Assay Federation Meetings Washington, DC
October 1977	Effects of Lipid Perturbents on Adenylate Cyclase Components in Hepatic Plasma Membranes Federation Meetings Atlanta, GA

Abstracts

Effects of Lipid Perturbents on Adenylate Cyclase Components in Hepatic Plasma Membranes. P.M. Lad, M.S. Preston, A.F. Welton and M. Rodbell. Federation Proceeding, October 1977.

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Lad PM, Nielsen TB, Londos C, Preston MS and Rodbell M. Independent Mechanisms of Adenosine Activation and Inhibition of Turkey Erythrocyte Adenylate Cyclase Systems. *Journal of Biological Chemistry.* November 25, 1980;255:10841-10846.

Lad PM, Nielsen TB, Lin MC, Cooper DMF, Preston MS and Rodbell M. "Toward a Unifying Hypothesis for the for the Effects of Cholera Toxin-Catalyzed ADP-Ribosylation in Diverse Adenylate Cyclase Systems" in Novel ADP-Ribosylation Regulatory Enzymes and Proteins. (Sugimura T and Snulson M, eds.) Elsevier-North, Holland Press, New York; 1980.

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Londos C and Preston MS. Activation of the Hepatic Adenylate Cyclase System by Divalent Cations. A Reassessment. *Journal of Biological Chemistry.* September 10, 1977;252:5957-5961.

Londos C and Preston MS. Regulation of Glucagon and Divalent Cations of Inhibition of Hepatic Adenylate Cyclase by Adenosine. *Journal of Biological Chemistry.* September 10, 1977;252:5951-5956.

III. Background

A. Alpha Therapeutic Corporation Products and Current Manufacturing Practices

The manufacturing practices of Alpha Therapeutic Corporation for fractionating plasma products are regulated and approved by the Food and Drug Administration. All improved manufacturing processes have been implemented in an effort to provide safer and more efficacious products for our recipients. The following table represents manufacturing modifications by the Alpha Therapeutic Corporation that were made to improve viral inactivation:

Table 1. Process improvements for Alpha's Products

Product	Virus Inactivation Method Change	Date Approved
Albumin, (Human) Albutein® Plasma Protein Fraction (PPF) Plasmatein®	Heat Treatment (Pasteurization)	1951
Factor VIII (AHF) Profilate® Alphanate®	Heat Treatment Solvent Detergent Dual Viral Inactivation (Solvent Detergent/Heat Treatment)	1984 1990 1997
Factor IX (CFN) Profilnine® AlphaNine®	Heat Treatment in n-heptene slurry Heat Treatment in n-heptane slurry Solvent Detergent) Dual Viral Inactivation (Virus filtered/ Solvent Detergent)	1984 1990 1992 1996
IGIV Venoglobulin®	Solvent Detergent	1991

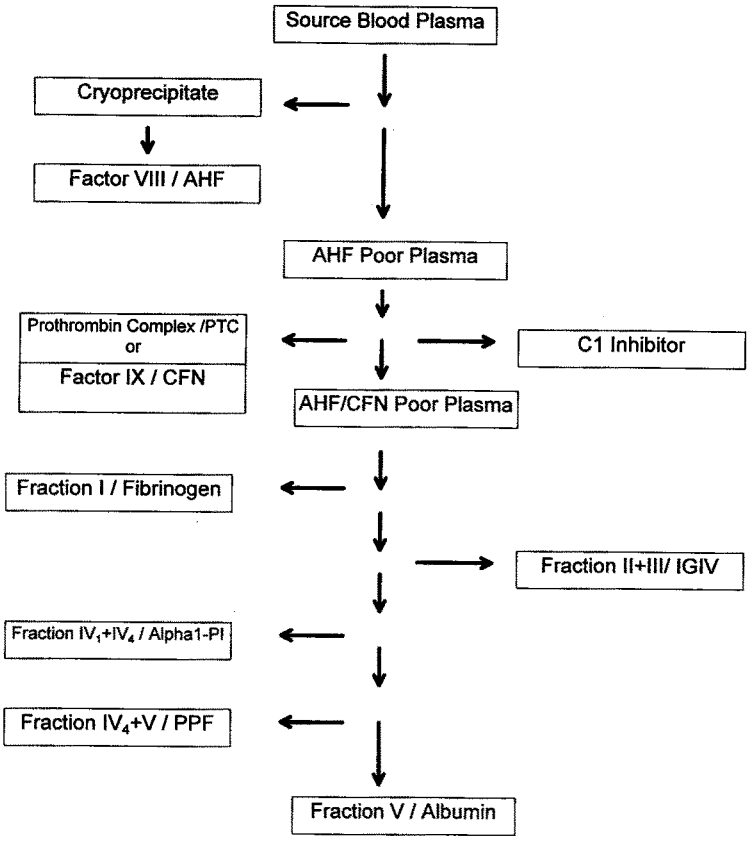
Plasma Processing

Human plasma is the raw material that is the basis for all of our licensed products. Alpha Therapeutic Corporation currently owns or has contracts with nearly 100 donor centers. Each of the donor centers utilizes a process called autopheresis that separates the human plasma from whole human blood. The autopheresis procedure allows for the plasma to be collected into a single collection bottle with an integral testing tube attached to the bottle. Then the autopherisis procedure allows the blood donor's red and white blood cells along with the their platelets to be safely returned into their body. The plasma in the collection bottle is stored in a freezer while the sample is sent to the Alpha Therapeutic Corporation Memphis Laboratory for viral marker testing. The plasma is then sent to the Alpha Therapeutic Corporation's Quarantine Storage Warehouse until a suitable time has elapsed and each unit of plasma can be 100% inspected for quality.

Manufacturing Plasma Pools

Alpha Therapeutic Corporation's products are derived from fractionating or refining pools of human plasma into different proteins and immunoglobulins. There are over 100 proteins in plasma from healthy individuals. Generally, proteins are removed in a certain order dependent on biological and chemical processing considerations. Plasma is thawed and centrifuged into cryoprecipitate which is purified into Antihemophilic Factor (AHF) of Factor VIII. Plasma with the AHF removed (AHF poor plasma) is then separated into Prothrombin Complex (PTC) or Coagulation Factor IX (CFN). The remaining material can be processed into either immunoglobulins, Plasma Protein Fraction (PPF) and/ or Albumin. Alpha Therapeutic Corporation is also developing purification processes that will allow us to provide the Fibrinogen, Alpha 1-Proteinase Inhibitor and C1- Esterase inhibitor for clinical trials. All of our products are administered intravenously. A simplified flow diagram has been provided on the next page which demonstrates the entire process of manufacturing the proteins and immunoglobulins from human plasma.

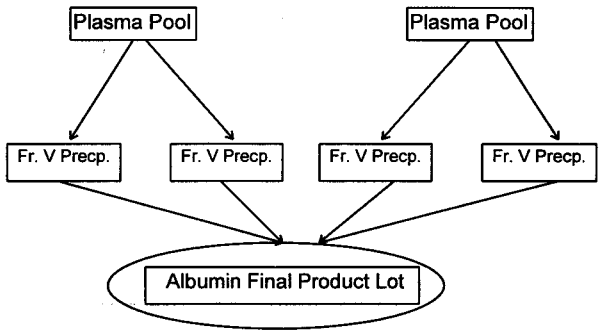
Alpha Therapeutic Manufacturing Flow Chart



Albumin Process

The albumin fractionation process is based on Dr. Edwin Cohn's cold alcohol fractionation/purification process[1,2]. As previously explained, the process of manufacturing Albumin (Human) is an integral part of the entire manufacturing system. Looking at the preceding manufacturing flow diagram, the amount of albumin actually produced is only one product of many from the same plasma donors. As each of the different products are removed from the plasma, a precipitate or paste remains. These batches of paste occur throughout the entire manufacturing process and are defined as intermediate products. In order to maximize the output yield and efficiently utilize the fractionation equipment for processing, purifying, and virus inactivation, it is necessary to combine different batches of the intermediate products before they are further processed. As illustrated in the simplified albumin batch process diagram below, it is necessary to have two starting batches of plasma to make four batches of intermediate product which are finally processed to make a single lot of final product. After the product has been purified, Albutein® and Plasmatein® are heated at 60 °C for ten hours.

Simplified Albumin Batch Process

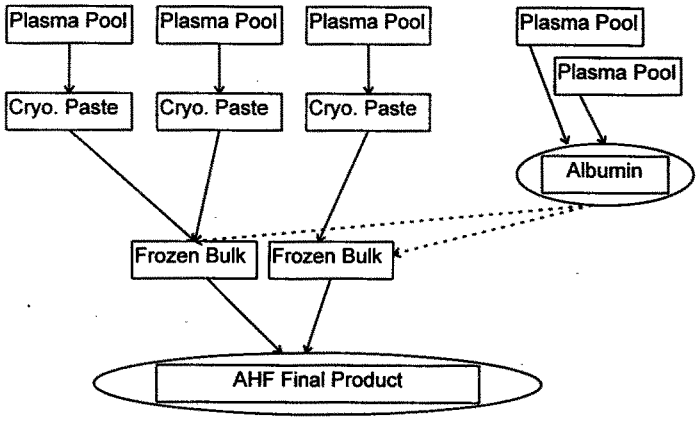


AHF Process

Alphanate® is prepared from pooled human plasma by cryoprecipitation of the Factor VIII, fractional solubilization and further purification employing heparin-coupled, cross-linked agarose which has an affinity to the heparin binding domain of vWF/FVIII:C complex. The product is treated with a mixture of tri(n-butyl) phosphate (TNBP) and polysorbate 80 to reduce the risks of transmission of viral infection. In order to provide an additional safeguard against potential non-lipid enveloped viral contamninants, the product is also subjected to an 80 °C heat treatment for 72 hours. The Factor VIII (AHF) manufacturing process also utilizes the combining of different intermediate batch products. In addition to the intermediate batch products being combined, final container Albumin is added to the product as a stabilizer. If the Factor VIII process includes three plasma lots as starting material and the Albumin process has two plasma lots as starting material, then there would be a minimum of five plasma lots or pools required to make the AHF product. The illustration below has been simplified and does not include all of the processes and intermediate products for the final product.

Another important consideration for the processing of AHF is that a large, companion protein, von Willebrand Factor, vWF is co-purified with the Factor VIII. This process is not included in recombinant DNA or monoclonal antibody purified Factor VIII products. Von Willebrands' disease, caused by deficiencies in vWF, is an inherited bleeding disorder affecting both males and females. Alphanate® with vWF Factor VIII complex is in clinical trials to test treatment of this disorder.

AHF Simplified Batch Process



Prothrombin Complex (PTC) Process

Profilnine® is a non-activated Factor IX Complex (PTC) prepared from pooled human plasma and purified by DEAE cellulose adsorption. Profilnine® is treated with a mixture of the organic solvent tri(n-butyl)phosphate and the nonionic detergent polysorbate 80 (Solvent Detergent Mixture) to reduce risks of transmission of viral infection. The (PTC) process utilizes similar batch processing as described for Albumin and AHF.

CFN Process

Alphanine® , Coagulation Factor IX (Human), is a highly purified, solvent detergent treated, virus filtered preparation of Factor IX derived from human plasma. The CFN process also utilizes similar batch processing as described for Albumin and AHF.

IGIV Process

Immune Globulin Intravenous (Human), Venoglobulin®-S Solution Solvent Detergent Treated, is a sterile, highly purified solution of intact unmodified human immunoglobulin G intend for intravenous use. Immunoglobulin G is isolated from human plasma by Cohn-Oncley cold alcohol fractionation and ion exchange chromatography. The manufacturing process includes treatment with a mixture of tri-n-butyl phosphate (TNBP) and polysorbate 80. The IGIV process is much more complex with many more intermediate processes required to purify the final product. It is also ensures that the antibodies come from a donor population with geographic diversity in order for the product to provide broad spectrum antibodies. The IGIV is also stabilized with Albumin and thus a higher number of donors will be normally included in the manufacturing of this product.

Critical Proteins

When processing Source Plasma, the dosage of each of the critical proteins should be considered. The levels of these critical proteins in one unit of Source Plasma purify to 0.03 doses of Factor VIII, 0.07 doses of Factor IX, 0.05 doses of IGIV and 0.82 doses of Albumin.

III. Background

B. Summary of Pool Sizes and Donor Exposure Related Final Products

The manufacturing records for final products were reviewed for the months of April through June of 1997 and the number of donors associated with each of the final products was estimated. Because it is difficult to actually determine the number of unique donors in each manufacturing pool, the conservative estimate of one donor for every unit of plasma was assumed. It was then determined which plasma pools contribute to each of the intermediate products and which of the intermediate products went into each of the final products. A summary of the actual manufacturing production lots has been provided below for each of Alpha Therapeutic Corporation's licensed products. Please note that final container Albumin is added in the manufacturing of both AHF and IGIV as stabilizer and the number of donors associated with the Albumin lots has been estimated and included to show the true association of donors to final container lots.

During the last six months there were no final products produced from Recovered Plasma, plasma collected from whole blood donors. Alpha may elect to use Recovered Plasma, as permitted by our approved product licenses, in the future.

Table 2. Range of Donors Associated with Manufacturing of the Final Product Lots

ATC Product	Range of Donors Associated with the Manufacturing of the Final Product Lots (ATC May -June 97)
Albumin (5%)	5,978 - 68,544 donors
Albumin (25 %)	10,482 - 102,879 donors
Factor VIII	63,228 - 98,294 donors
Factor IX	22,317 - 33,119 donors
IGIV	75,543 - 125,637 donors

III. Background

C. Manufacturing Considerations for Reducing Plasma Pool Size

Alpha Therapeutic Corporation is currently manufacturing at full capacity and reducing the number of donors associated with the final products would have an immediate effect on the output of the final product. Because less plasma would be processed, less product would be made. The following table represents estimates for the loss of output for the active ingredient for each of Alpha's products:

Table 3. Percentage of Final Product Lost when Reducing Manufacturing Scale

Product	60,000 donor pool limit	15,000 donor pool limit	5,000 donor pool limit
Albumin	5%	10%	20%
Factor VIII (AHF)	5 - 10%	43%	68%
Factor IX (CFN)	0%	50%	50%
IGIV	5 - 10%	31%	41%

Alpha Therapeutic Corporation is committed to voluntarily limit our final product donor exposure to the 60,000 limit with minimal yield losses by the end of 1997. As discussed in the previous sections all of the processes are interrelated and reducing plasma input would effect the production cycles and schedules for all of the products. Moving to the lower limit of 15,000 donation exposures or 5,000 donation exposures would require extensive capitalization and construction in order to prevent output loss. The amount of land, equipment, utilities and human resources would have to be increased before full production could be resumed. All of the manufacturing expansions would need to be planned, validated and then an extensive establishment license application procedure with the FDA would need implementing. Changes of this magnitude in a pharmaceutical manufacturing industry normally require a minimum of five years before license approval can be obtained.

IV. SAFETY AND PLASMA DERIVATIVES

A. Safety Related to the Size of Plasma Pools

The issue of safety as related to the size of plasma pools was first expressed at the March 1995 meeting of the Blood Products Advisory Committee when Dr. Lynch presented the results of his modeling on the risk to one-time and chronic recipients with agents of different prevalence and the size of the plasma pools. It should be noted that a more precise term for reduction in plasma pool size should be the donor exposure in a final container lot. We have noted the various presentations made by FDA, consumer group representatives and industry officials may be inconsistent with respect to the term pool size reduction. At one point in the earlier discussions, pool size reduction was interpreted to mean the reduction of the plasma pooling operations, with no impact on the combination of intermediates during the purification processes or the addition of Albumin (Human) as a stabilizer as described in the previous section. To prevent confusion, the reduction in donor exposure will be discussed in our written testimony. To be absolutely clear, donor exposure is different from donation exposure. Donor exposure counts the number of donors in a final container lot. Donation exposure would count every donation in a final container lot even if the purified protein from several donations from the same individual were included in the final container lot. For the record, Alpha Therapeutic Corporation does not concur with the FDA's proposal that there should be two standards, one for Source Plasma and a different standard for Recovered Plasma.

Let us review the concern of safety with respect to donor exposure. Dr. Lynch from the Office of Blood Research and Review published an article[3] in *Transfusion* which mathematically modeled the risk of exposure to a pathogenic agent when donor numbers increased and the prevalence of the infectious agent varied. The author makes a statement that is the foundation for our testimony "Compared to other measures, such as donor screening and deferral and virus inactivation or removal during processing, restricting the manufacturing scale would be a relatively ineffective measure for improving safety." The only case when limiting donor exposure may be marginally beneficial is for one-time recipients for the emergence of an unknown infectious agent with extremely low prevalence for which the current manufacturing processes do not inactivate or remove that agent. As an example, Dr. Lynch and his co-authors calculate that for an agent with a prevalence of 1 in one million, a maximum donor exposure of 10,000 donors would yield a 1 in 100 chance of exposure to that agent for a one-time recipient. Is a 1% risk an acceptable level of risk? The number of independent infusions to obtain a 100% risk of exposure for the lifetime recipients of plasma derivatives is very quickly reached and is not significantly decreased by reducing donor exposure. The prevalence of CJD

is probably in the order of 1 in 50,000. Table 1 from the *Transfusion* article is partially reproduced below to illustrate the marginal effects of reduction of donor exposure:

Table 1. Effect of manufacturing scale on risk of exposure

Scale of manufacturing	Number of independent infusions		
(number of donors)	1	10	100
Prevalence of agent = 1 in 500,000			
60,000	11%	70%	100%
25,000	5%	39%	99%
10,000	2%	18%	86%
6,000	1%	11%	70%
2,500	0.5%	5%	39%
1,000	0.2%	2%	18%
Prevalence of agent = 1 in 50,000			
60,000	70%	100%	100%
25,000	39%	99%	100%
10,000	18%	86%	100%
6,000	11%	70%	100%
2,500	5%	39%	99%
1,000	2%	18%	86%

The authors of the paper made several assumptions in order to make any mathematical calculations. Two important assumptions were that (1) there was no difference in exposure and infectivity, and that (2) the process did not alter the exposure or infectivity. As stated by the authors, the risk of exposure does not necessarily equate to the risk of infection. Rather, in all cases, risk of exposure is greater than or equal to the rate of infection. Thus, this model presented provides worst-case estimates of risk. These assumptions can be made to allow for worst-case mathematical modeling but are not supported by actual data. A review of the most recent transmission of a viral agent by a plasma derivative will help illustrate that infectivity does not equate with exposure. The example is the transmission of hepatitis C by an Immune Globulin Intravenous (Human) preparation, Gammagard®. Dr. Bresee and his colleagues presented an analysis[4] of the seroconversion of recipients of Gammagard® showing that the risk of seroconversion for markers of hepatitis C increased with the number of HCV RNA-positive lots infused and the quantity of HCV RNA material infused. Importantly, not all recipients were infected as the highest percentage of seroconversions seen with the highest levels of HCV RNA infused did not exceed 30%. The reasons for not observing seroconversions in 100% of the recipients may, in part, be attributed to the fact that infectivity does not equate to exposure. There are many factors that contribute to the loss of infectivity - from the

recipient's susceptibility to the reduction of infectiousness related to steps in the manufacture of the product. **Furthermore, the data presented in this article support two more effective safety measures: (1) implementation of viral inactivation and removal methods in the purification process, and (2) implementation of polymerase chain reaction testing for HCV RNA in the final product.**

In consideration of the second assumption, the purification processes contribute to the elimination or reduction of infectious agents in the final product plasma derivatives. An example is reduction of human immunodeficiency virus during the Cohn-Oncley fractionation process as described in elegant experiments performed in government and industry laboratories.[5,6,7,8] Although these steps had been part of the purification process, the steps contributed significantly to the safety of the products as no cases of transmission of HIV through Albumin or Immunoglobulin products have been found. Other steps, added as specific virus inactivation steps, such as solvent-detergent treatment have inactivated or removed specific viruses and have been found subsequently to inactivate or remove other viruses. For instance, solvent-detergent treatment for coagulation products, studied in over 400 recipients, showed no evidence of infectious agent transmission.[9] Dr. Horowitz and colleagues present data for over 3.8 million doses of Factor VIII without transmission of lipid enveloped viruses such as HIV, hepatitis B or hepatitis C.

It is vitally important to consider and test the hypothesis before changing a validated manufacturing process. An example of a change postulated to be beneficial - that of screening plasma for fractionation for the absence of antibodies to hepatitis C virus - upset the delicate balance of safety for one of the immunoglobulin products. This example reminds us of the need to develop tests for antigens or viral particles; antibodies to pathogenic agents are desirable to complex viruses in the process and to protect recipients from infection, especially immunocompromised patients receiving immunoglobulins. Furthermore, Don Tankersley, in his presentation before the Blood Products Advisory Committee in December 1996, described his concerns that reducing donor exposure through reducing the manufacturing scale may have the opposite effect of decreasing safety.[10] He cites the effect of dilution, and the presence of neutralizing or complexing specific antibodies or fortuitous antibodies as factors that could be detrimentally affected by reductions in manufacturing scale.

Alpha Therapeutic Corporation has already implemented practices to limit the number of donors represented in our final container lots to not more than 60,000 donors. These practices are in compliance with Current Good Manufacturing Practices and assure the consistency of our production methods. Some of the manufacturing scales for our products are well below the limit stated above and will remain so. We remain committed to working with regulatory authorities to constantly enhance our margin of safety through implementing the best measures as early as possible. However, we can not condone the change of validated methods without careful consideration of the consequences for both product safety and efficacy.

IV. Safety and Plasma Derivatives

B. Safety and Donor Selection

Source Plasma

All human plasma collected for the manufacture of plasma derivatives marketed by Alpha Therapeutic Corporation meets the requirements of the US Code of Federal Regulations. These regulations include performing a physical, obtaining a medical history, determining donor health status by vital signs, total protein, hematocrit and periodic testing for syphilis (RPR) and Serum Protein Electrophoresis (SPE). Donors, who exhibit "High Risk Behavior" for AIDS and CJD are also excluded.

Alpha Therapeutic Corporation has voluntarily implemented higher standards for donor requirements which involve participating in the American Blood Resource Association Quality Plasma Program (QPP). The QPP standards require donor centers to check a National Donor Deferral Registry before allowing a donor to participate. Donors are also screened for drugs and they are required to be a member of the community. In addition to the QPP standards, Alpha takes an extensive medical history from every new donor and as an additional precaution against CJD risk, Alpha will not take donors older than 60 years and will not take donors who have had a cornea transplant. Both of these exclusions reduce the risk of collecting from a donor who is at risk for CJD. Alpha accepts plasma for manufacturing from only "Qualified Donors" that have had a minimum of two donations with all negative viral marker tests.

Plasma Collection Facilities

Source Plasma (Human) is collected at US Food and Drug Administration (FDA) licensed plasmapheresis facilities. Alpha Therapeutic Corporation (ATC) currently owns and operates *63* licensed plasmapheresis establishments for the collection of Source Plasma (Human). Some plasma may also be obtained from other US FDA licensed plasmapheresis establishments contracted by ATC. All Source Plasma (Human) is collected in the United States and all plasmapheresis establishments are subject to periodic inspection by the US FDA to ensure compliance with the Code of Federal Regulations (CFR).

All of the centers used for the collection of plasma are Quality Plasma Program (QPP) certified and periodically monitored by the American Blood Resources Association.

All donations are screened at an FDA inspected and licensed central testing laboratory owned and operated by ATC.

Data on Epidemiology of Blood-Borne Infections

Plasma is not collected from areas or locations with high risk donor populations.

All of the centers used for the collection of plasma are Quality Plasma Program (QPP) certified and periodically monitored by the American Blood Resources Association (ABRA). ABRA monitors viral marker rates and has established a QPP standard that each center must meet before certification can be issued.

An ongoing evaluation of the epidemiology at the ATC and contract collection centers is conducted monthly. Results of Hepatitis B Surface Antigen (HBsAg), HIV-1 antigen, antibodies to Human Immunodeficiency Virus Types 1 and 2, and antibodies to Hepatitis C Virus (HCV) testing are included in the epidemiology statistics evaluated.

Nature of the Examination and Interview of Donors

Each center, in accordance with the FDA approved procedures screens all donors to ensure that each person is in good health and that the plasma collected is not from a high risk population for blood-borne infections. This includes examination for signs and increased risk of Acquired Immunodeficiency Syndrome (AIDS). A review of the donor's medical history is conducted with a series of questions to determine any affiliations with groups known to be at greater risk for contracting AIDS. Copies of the donor examination form and screening questions follow on the next two pages. Both of these forms have been reviewed and approved by the FDA. These examinations and donor interviews are conducted in a voluntary self-exclusion confidential setting.

	1		2		3		4		5		6		7		8		9		10	
	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
lave you read and do you understand the ear Donor Letter given to you regarding cquired Immune Deficiency Syndrome IDS)? (Yes answer is OK.)																				
lave you ever participated in any of the ehaviors identified to be at increased sk for AIDS listed on this poster?																				
HAVE YOU EXPERIENCED THE FOLLOWING?:	1		2		3		4		5		6		7		8		9		10	
	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
t any time: AIDS; received any human derived ssue (e.g. human pituitary derived substances, uman growth hormone - hGH, corneal ansplants) or Tegison; history of viral hepatitis aundice) at the age of 11 yrs or later?																				
t any time: epilepsy, convulsions, fainting, eurosurgery; heart disease?																				
t any time: Blood disease, blood clotting oblems, hemophilia?																				
the past 12 months: Tattoo, acupuncture, erced body parts; close contact with hepatitis, ceived Hepatitis B Immune Globulin or hepatitis vaccine; received blood transfusion, blood omponent/derivative or tissue transplant; carcerated?																				
the past 8 weeks: given or lost whole blood or BC's?																				
ecent: Surgery (Major-3 mo.) (Minor-1 mo.); egnancy, tooth extraction; shots, treatments, edications; under physician's care; Accutane or oscar (1 mo.)?																				
urrent: Respiratory disease: asthma, allergies, ayfever, cold or sore throat; boils, skin infection; ddicted to or under the influence of drugs or cohol?																				
Inexplained needle marks?																				
E: "No" for 1 or any "yes" answers for 2-10 l be explained in the "Remarks" column.	1	DATE	2	DATE	3	DATE	4	DATE	5	DATE	6	DATE	7	DATE	8	DATE	9	DATE	10	DATE
larity being plasmapheresed and agree that this blood and/or derivatives from it may be used in any manner decided by the plasma center. I also declare and certify under penalty of perjury that to the best of my knowledge I do not have any signs or symptoms suggestive of AIDS and that I am not a member of any High Risk group for AIDS. I accept the cash fee in full settlement of services rendered. He respuesto a las preguntas de arriba y son verdad según (yo) sé. Atestigo que voluntariamente estoy siendo plasmaferezado y estoy en acuerdo que ésta sangre y sus derivados se pueden usar de cualquier modo el centro de plasma quiere. Atestigo y declaro bajo pena de perjurio que no tengo cualquiera de las señas o sintomas sugestivo del SIDA y no soy un miembro de cualquiera de los grupos al riesgo elevado del SIDA. Acepto este honorario como pago entero por los servicios dados.	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY	SIGNATURE OF DONOR	INTERVIEWED BY

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HIV HIGH RISK QUESTIONNAIRE

YES	NO		
☐	☐	1.	**Do you have AIDS, or have you ever had a positive test for the AIDS virus (HIV)?**
☐	☐	2.	**Have you ever taken illegal drugs with a needle, even one time?**
☐	☐	3.	**Have you taken clotting factor concentrates for a bleeding disorder such as hemophilia?**
☐	☐	4.	**At any time since 1977, have you taken money or drugs for sex?**
☐	☐	5.	**Male donors: Have you had sex with another man, even one time since 1977?**
☐	☐	6.	**Female donors: In the last 12 months have you had sex with a man who had sex, even one time since 1977, with another man?**
☐	☐	7.	**Have you had sex in the last 12 months with anyone who has AIDS or has had a positive test for the AIDS virus?**
☐	☐	8.	**Have you had sex in the last 12 months with anyone who has ever taken illegal drugs with a needle?**
☐	☐	9.	**At any time in the last 12 months, have you given money or drugs to anyone to have sex with you?**
☐	☐	10.	**At any time in the last 12 months, have you had sex with anyone who has taken money or drugs for sex?**
☐	☐	11.	**Have you had sex in the last 12 months with anyone who has taken clotting factor concentrates for a bleeding disorder such as hemophilia?**
☐	☐	12.	**In the last 12 months, have you had syphilis or gonorrhea, or have you been treated for syphilis or gonorrhea?**
☐	☐	13.	**In the last 12 months, have you received blood or blood products by transfusion for any reason, such as an accident or surgery?**
☐	☐	14.	**Were you born in or have you lived in any of the following countries since 1977: Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, or Nigeria?**
☐	☐	15.	**If you have traveled to any of the above countries since 1977, did you receive a blood transfusion or any medical treatment with a product made from blood while you were in those countries?**
☐	☐	16.	**Have you had sexual contact with anyone who was born in or lived in the above countries since 1977?**
☐	☐	17.	**In the last 12 months, have you been an inmate of correctional institutions (including jails and prisons) or been incarcerated for more than 72 consecutive hours?**

_____
Donor Name

Initials of person obtaining history:_____ Date: _____

Quarantine Period and Procedures

Alpha Therapeutic Corporation, in participation with the industry has voluntarily agreed to hold plasma in quarantine for not less than 30 days. This quarantine will be extended for not less than 60 days by the end of 1997. This quarantine adds another layer of safety in that a donor's plasma can be retrieved from manufacturing should the donor subsequently test positive for a viral marker screen or new information is gathered concerning the safety of that plasma. This allows for a significant reduction in viral bioburden levels as the period immediately prior to antibody seroconversion is usually the period with high virus levels in plasma for blood borne-pathogens. For HIV the window period between infection and seroconversion is thought to be 22 days. For the HCV virus this seroconversion period is thought to be 98 days and for 56 days for HBV.

All units of Source Plasma (Human) collected by ATC and the contract centers are held in quarantine until each unit is visually inspected and each unit is verified with the test record to be non-reactive. Only after this inspection and all screening tests are acceptable can any plasma be released for manufacturing.

IV. SAFETY AND PLASMA DERIVATIVES

C. Safety and Plasma Testing

All tests for markers of infection for all Source Plasma donations collected by ATC and contract plasmapheresis centers are performed at Alpha Therapeutic Corporation's FDA licensed testing facility located at 5700 Pleasant View Road, Memphis, Tennessee 38134.

Tests and Specifications

Individual Donations

A sample of plasma is taken from each donor at the time of each donation and tested for the presence of Hepatitis B surface antigen (HBsAg), antibodies to Hepatitis C (Anti-HCV), HIV-1 antigen and antibodies to HIV Types 1 and 2 (Anti-HIV). Donors with repeatedly positive test results are rejected from further donations. The positive unit and all previous donation plasma units not pooled for manufacture in the preceding twelve months are retrieved and the plasma cosignees are notified according to federal regulations.

The plasma sample is also tested for the level of the liver enzyme alanine aminotransferase (ALT). ALT may be an indicator of liver disease and or a viral infection. Plasma units with unacceptable ALT levels are not acceptable and are subsequently dispositioned according to ATC written procedures. The donors are also deferred from donating.

Alpha Therapeutic Corporation continuously explores the most sensitive and specific test methodologies for indication of health status. We collaborate with other diagnostic kit manufacturers to study these test kits. We encourage test for antigens and viral nucleic acids rather than antibodies as removal of antibodies may be detrimental to the safety margin of our products. Furthermore, the presence of antibodies to specific viral antigens may be important in the prevention of disease in immunocompromised patients when treated with immunoglobulin preparations.

Individual plasma donations are tested as follows in the table found on the next page:

Table 4. Testing Algorithm for Testing Plasma Donations

Test / Frequency	Initial Test Result	Action Taken	Retest Result	Action Taken
HIV-1/2 Antibody (every donation)	Non-Reactive	Accept donation	N/A	N/A
	Reactive	Retest in duplicate	both duplicates HIV-1/2 Non-Reactive	accept donation, possible QA investigation
			one or both duplicates Reactive	permanent rejection of donor
			HIV-1/2 Reactive, HIV-1 Western Blot Positive	permanent rejection of donor
			HIV-1/2 Reactive, HIV-1 Western Blot Indeterminate or Negative, HIV-2 Non-Reactive	permanent rejection of donor
			HIV-1/2 Reactive, HIV-1 Western Blot Indeterminate or Negative, HIV-2 Reactive	permanent rejection of donor
HIV-1 p24 Antigen (every donation)	Non-Reactive	Accept donation	N/A	N/A
	Reactive	Retest in duplicate	one or both duplicates Reactive	permanent rejection of donor
			both duplicates Non-Reactive	accept donation, possible QA investigation
HBsAg (every donation)	Non-Reactive	Accept donation	N/A	N/A
	Reactive	Retest in duplicate	one or both duplicates Reactive	permanent rejection of donor
			both duplicates Non-Reactive	accept donation, possible QA investigation
HCV Antibody (every donation)	Non-Reactive	Accept donation	N/A	N/A
	Reactive	Retest in duplicate	one or both duplicates Reactive	permanent rejection of donor
			both duplicates Non-Reactive	accept donation, possible QA investigation
ALT (every donation)	< 2 x Upper limit of normal range	Accept donation	N/A	N/A
	≥ 2 x Upper limit of normal range	Reject donation	N/A	N/A
RPR (Qualitative) (for new donors and every four months)	Non-Reactive	Accept donation	N/A	N/A
	Reactive	Retest in duplicate	one or both duplicates Reactive	donor deferred
			both duplicates Non-Reactive	accept donation, possible QA investigation
SPE	Abnormal	Donor Deferred	N/A	N/A

All tests of individual donations are performed at the Memphis facility in accordance with the manufacturer's instructions.

Mini-Pool Testing

As an added precaution against the inclusion of any Source Plasma that may contain undetectable HIV virus, Alpha Therapeutic Corporation performs mini-pool testing for the HIV antibody.

Each donation tested according to the previous section is further checked for anti-HIV in supplementary testing. This testing utilizes "mini-pools" derived from samples of 64 donations and an alternative test kit to that is for testing individual donations. If this derived mini-pool of 64 samples tests reactive for anti-HIV, then the reactive sample(s) are identified by individual sample testing, using the alternative test kit, according to the scheme given in the previous table.

Plasma units corresponding to test samples that are confirmed reactive for anti-HIV at individual sample testing are rejected. The donors

associated with the reactive unit are deferred in accordance with the FDA guidelines.

A summary of the mini-pool sample testing is given in the following table:

Table 5. Testing Algorithm for Mini-Pools

Test Frequency	Initial Test Result	Action Taken	Retest Result	Action Taken
HIV Types 1 and 2-1/2 Ab/ (64 sample mini-pools)	Non-Reactive	Accept all mini-pooled units for fractionation		
	Reactive	Retest individual samples	Reactive sample(s) identified	Reject reactive donation(s) Accept non-reactive donations
			Reactive sample(s) not identified	Segregate all mini-pooled units pending further tests/ investigations

Testing of mini-pools is performed at the Memphis facility

PCR Testing

Alpha Therapeutic Corporation has received permission from the FDA and has begun clinical trials on the ability of the most sensitive test available, polymerase chain reaction (PCR) in mini-pools to detect viral nucleic acid material from both the HIV and HCV virus. The PCR method for testing requires that a sample of pooled plasma be taken, then processed such that if there is any of the virus's genetic code within the sample it will multiply to detective levels.

Early in infection, the level of viremia is normally at its highest and antibodies have not been produced in detectable amounts. The period of time in which the donor has been infected by a virus but the antibodies cannot be detected by normal diagnostic testing is known as the window period. Because PCR testing detects at the virus genetic level, donors can be identified in days or even months sooner than if only the traditional test is performed and thus closing the window period.

HISTORY AND PHYSICAL EXAMINATION FORM

DONOR NAME_____AGE ____ SEX ____ ID# _____

C _____

HISTORY

	YES	NO
HEART DISEASE		
LUNG DISEASE, ASTHMA, EMPHYSEMA		
HEPATITIS OR EXPOSURE TO HEPATITIS REQUIRING AN INJECTION, JAUNDICE, LIVER DISEASE		
HIGH BLOOD PRESSURE		
DIABETES MELLITUS		
KIDNEY DISEASES		
SKIN DISEASES		
RISK FACTORS FOR CJD: FAMILY HISTORY OF DEMENTIA? CORNEAL TRANSPLANTS? NEUROSURGERY? HUMAN PITUITARY DERIVED SUBSTANCES?		

	YES	NO
PREGNANCY		
ALCOHOLISM		
DRUG ADDICTION		
VENEREAL DISEASES		
CANCER		
MALARIA		
EPILEPSY		
BRUCELLOSIS (CALIF. ONLY)		

	YES	NO
ALLERGIES		
WEIGHT LOSS *		
SWOLLEN GLANDS		
FREQUENT DIARRHEA		
UNDER DR. CARE		
MEDICATIONS		
SURGERY		
BLOOD TRANSFUSION		

COMMENTS: (* IF WEIGHT LOSS, HOW MUCH OVER WHAT PERIOD OF TIME?) _____

_____

_____

PHYSICAL EXAMINATION

BP: _____ PULSE: _____ WT: _____

GENERAL APPEARANCE _____

H.E.E.N.T. _____

NECK _____

HEART _____

LUNGS _____

CHEST _____

ABDOMEN _____

EXTREMITIES _____

LYMPHATICS _____

NEUROLOGIC _____

PSYCHIATRIC _____

URINALYSIS: GLUCOSE _____ PROTEIN _____ URINALYSIS BY: _____

A TED ☐ REJECTED ☐

EXAMINER'S SIGNATURE _____

Manufacturing Pools / Sub Pools

All cryo-poor plasma pools are tested by Alpha Therapeutic Corporation utilizing the most recent generation of FDA licensed test kits intended for the detection of antibody (HCV and HIV Types 1 and 2) or antigen (HBV) in donations of human serum or plasma. Additionally, all cryo-poor plasma pools will be tested using an alternate HIV antibody test kit.

Testing is performed at Alpha Therapeutic Corporation's on-site laboratory located at our manufacturing facility, 5555 Valley Boulevard, Los Angeles, California, USA.

Test kits in used are as follows:

Table 6. Test Kits Currently Used by Alpha Therapeutic Corporation

Test Description/ Manufacturer	Generation	FDA License Number	Test Used For Screening donations	mini-pools	mfg. pools
Genetic Systems HIV-1/HIV-2 EIA 1/2 EIA	second	978	✓	NA	✓
Abbott HIV-1/HIV-2 (rDNA) 1/2 EIA	third	043	†	✓	✓
Cambridge Biotech HIV-1 Western Blot	first	1063	‡	NA	†
Genetic Systems HBsAg EIA 2.0	third	978	✓	NA	✓
Abbott AUSRIA® II-125 Antibody to Hepatitis B Surface Antigen ^{125} I (Human)	third	043	✓	NA	†
ORTHO HCV 3.0 ELISA	second	156	✓	NA	✓

† If indicated by test results for mini-pools or pools.
‡ If indicated by EIA result

System to Track the Path of any Donation

ATC has a numbering system in place which enables the path taken by each donation to be traced from its origin (the donor) through to the finished products. Each donation collected from each donor is assigned a unique alphanumeric code (bleed number) which is associated with the record of plasma collection, the labeling of the unit of Source Plasma, the labeling of any plasma test samples, all documentation of test results, and the manufacturing records for finished products.

For all viral marker testing, each center must check their records and report twelve months of look-back information (identification of any plasma units that originally tested negative for a viral marker but collected from a donor who subsequently tests positive for a viral marker). All of the units of plasma not yet issued to a manufacturing pool are segregated, quarantined and subsequently dispositioned according to FDA guidelines. Any consignees of affected Source Plasma are notified of the donor's current status.

IV. SAFETY AND PLASMA DERIVATIVES

D. Safety and Virus Inactivation

Alpha Therapeutic Corporation (ATC) recognizes that there is not any current technology to ensure that all pools of plasma may be free of all known and unknown viral contamination. Therefore, ATC has developed, improved and continues to seek new methodology and technology to inactivate virus during the manufacturing process. ATC participates with an industry consortium to share new technologies and communicates with experts in the fields of virus inactivation to find better ways to remove viruses of all types.

The FDA mandates the viral inactivation step of heat treatment (pasteurization) for albumin products. Alpha has also developed a method for heat treating the Factor VIII during the manufacturing process which is effective in virus inactivation. Alpha also uses a solvent detergent process to inactivate viruses such as HIV, HBV and HCV very efficiently. Other viruses such as hepatitis A and the parvovirus appear to be more resilient and so Alpha has developed nanofiltration to remove the viruses bases on size. In additional to these methods, the actual manufacturing process of partitioning the proteins from the plasma also assists with Alpha's goal of maximum virus removal. The following charts summarize the effects of Alpha's virus inactivation during manufacturing of the products.

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Table 7. Summary of Viral Inactivation/Removal Steps for Albumin (Human)

Removal Step	Virus (Reduction)					
	HIV-1	HIV-2	BVD (HCV)	IBR (HBV)	EMC (HAV)	PPV (B-19)
Pasteurization, 5%	≥8.8	N/D	≥5.9	≥6.7	≥6.5	N/D
Fraction IV Filtration	N/D	N/D	3.5	≥5.6	5.4	3.2
Total Log Removal	**≥8.8**		**≥9.4**	**≥12.3**	**≥11.9**	**3.2**

Removal Step	Virus (Reduction)					
	HIV-1	HIV-2	BVD	IBR	EMC	PPV
Pasteurization, 20%	N/D	N/D	≥6.9	≥6.9	≥6.0	N/D
Fraction IV Filtration	N/D	N/D	3.5	≥5.6	5.4	3.2
Total Log Removal			**≥10.4**	**≥12.5**	**≥11.4**	**3.2**

Removal Step	Virus (Reduction)					
	HIV-1	HIV-2	BVD	IBR	EMC	PPV
Pasteurization, 25%	≥7.9	6.5	≥6.9	≥6.8	≥6.0	N/D
Fraction IV Filtration	N/D	N/D	3.5	≥5.6	5.4	3.2
Total Log Removal	**≥8.3**	**6.5**	**≥10.4**	**≥12.4**	**≥11.4**	**3.2**

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Table 8. Summary of Viral Inactivation/Removal Steps for Alphanate

Removal Step	Virus (Reduction)								
	HIV-1	HIV-2	VSV	Sindbis (HCV)	BHV (HBV)	Polio (HAV)	CPV (B19)	BVD (HCV)	HAV
3.5% PEG precip	≤1.0	N/D	N/D	N/D	≤1.0	3.3	1.2	≤1.0	N/D
Solvent/Detergen	≥10.0	≥6.0	≥6.8	≥6.4	≥8.0	N/D	N/D	≥4.5	N/D
Heparin Column	2.0	N/D	N/D	N/D	7.6	≤1.0	≤1.0	≤1.0	N/D
Freeze Drying	N/D	N/D	N/D	N/D	1.3[a]	3.4[a]	≤1.0[a]	≤1.0[a]	1.9[a]
Dry Heat	N/D	N/D	N/D	N/D	2.1[a]	≥2.5[a]	4.1[a]	≥4.9[a]	≥6.1[a]
Total Log Removal	**≥12.0**	**≥6.0**	**≥6.8**	**≥6.4**	**≥19.0**	**≥9.2**	**5.3**	**≥9.4**	**≥8.0**

N/D = Not Done

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Table 9. Summary of Viral Inactivation/Removal Steps for AlphaNineSD and PTC-SD

Removal Step	Virus (Reduction)								
	Sindbis (HCV)	VSV	PPV (B-19)	HIV-1	HIV-2	EMC (HAV)	Reovirus	PRV (HBV)	HAV
DEAE Cellulose Chromatography	1.4	N/D	1.9	N/D	N/D	N/D	N/P	N/D	N/D
Solvent/Detergent	≥ 5.3	≥4.9	ND	≥12.2	≥6.0	N/D	N/P	≥5.6	N/D
Two Dextran Sulfate Silica Columns	4.7	N/D	2.6	N/D	N/D	N/D	N/D	N/D	N/D
Viresolve Filtration**	N/D	N/D	3.8	N/D	N/D	3.9	4.3	N/D	≥4.4
Total Log Removal	**≥11.4**	**≥4.9**	**8.3**	**≥12**	**≥6.0**	**3.9**	**4.3**	**≥5.6**	**≥4.4**

N/D = Not Done
**Not applicable to PTC-SD

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Table 10. Summary of Viral Inactivation/Removal Steps for Venoglobulin®S

Removal Step	Virus (Reduction)							
	HIV-1	HIV-2	Sindbis	VSV	Vaccinia	HCV	PPV	EMC
Solvent/Detergent	≥10.0	≥5.8	≥5.9	≥5.5	2.5	3.0	N/D	N/D
4% PEG Precipitatio	2.0	N/D	1.9	3.9	4.5	4.0	≥5.1	≥3.7
Bentonite Filtration	N/D	N/D	N/D	N/D	N/D	N/D	3.3	≥4.8
Cohn Oncley Ethanol Fractionatio	11.0	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Total Log Removal	**≥ 23.0**	**≥5.8**	**≥7.8**	**≥9.4**	**7.0**	**7.0**	**≥8.4**	**≥8.5**

N/D = Not Done

IV. SAFETY AND PLASMA DERIVATIVES

E. Safety and Product Testing

The FDA has mandated that before any final product lot can be released, it must be tested for sterility, potency, purity and safety. The final product must also be stable throughout its expected and approved shelf life. Therefore the product is periodically tested to be sterile, potent, pure, and safe for the duration of its shelf life.

Alpha Therapeutic Corporation has also decided that every product lot released should be tested one more time to ensure that there were no GMP errors during the testing of the plasma, testing of the pools, and during the manufacturing of the plasma. The following tests are also performed on every final product lot before it can be released for use:

Table 11. Voluntary Testing Performed on Final Products

Antibody Testing	Surface Antigen Testing	PCR Viral Testing
HIV HCV	HBV	HIV HAV HBV HCV

IV. SAFETY AND PLASMA DERIVATIVES

F. Safety and Patient Monitoring

Alpha Therapeutic Corporation continues to monitor the safety and efficacy of its licensed products through marketing surveillance and on-going clinical trials long after receiving FDA approval. We are actively involved with our recipients and their health care providers to supply current education on the risks and benefits of infusing our products.

Alpha Therapeutic Corporation monitors recipients of our products through the marketing surveillance system established and regulated by the FDA. This system asks recipients or health care providers to voluntarily report any adverse side effect associated with the administration of a pharmaceutical product. These side effects are usually reported to the company, but occasionally become known from information provided to the FDA or another company. These reports of adverse events are tracked and investigated as required by regulation and our established procedures. We report serious and unexpected adverse events to the FDA within 15 days. We evaluate our reports for increased frequency and provide periodic reports to the FDA with analyses of all marketing surveillance data. We cooperate fully with regulatory authorities around the world to investigate and take proper corrective actions to prevent unsafe administration of our products.

Alpha Therapeutic Corporation voluntarily continues on-going clinical trials of most of our licensed products to monitor the safety and efficacy of these products as we implement improved processes. We currently have on-going clinical trials for Alphanate®, AlphaNine®-SD, and Venoglobulin®-S. For some of our clinical trials, Albutein® has been infused as a placebo. During the course of these trials, we monitor recipients for acute adverse reactions, longer-term reactions and seroconversion to known viruses. To date, the results of our clinical trials support the safety of our currently marketed products.

Patient monitoring may be a very effective method to maintain our vigilance for newly emerging pathogenic agents. The Centers for Disease Control and Prevention (CDC) has funding to monitor the hemophiliac population for viral epidemiology. Alpha Therapeutic Corporation encourages the rapid expansion of this surveillance system. We met with CDC staff a year ago to lend our support and cooperation to this effort. The CDC is also conducting a study of hemophiliacs who have died recently to evaluate the risk of CJD transmission by Factor VIII concentrates. We pledge our cooperation with the CDC in their investigative efforts.

Alpha Therapeutic Corporation believes that the most likely population to succumb to a newly emerging pathogenic agent is recipients of untreated (not virally inactivated) blood components. We encourage the National Institutes of Health to support research into better detection methods and inactivation or removal steps for newly emerging agents. We encourage the CDC to conduct long-term surveillance studies with recipients of blood products. Part of these surveillance studies must include samples of blood from donors so that testing can confirm the transmission of a pathogenic agent.

Alpha Therapeutic Corporation supports a wide variety of educational efforts in the health care community on the proper usage of our products as well as their risks and benefits. We supply educational materials to health care providers for patients. We provide medical staff to handle questions and emergencies. We present the results of our research at scientific and medical meetings and in scientific journal articles. More importantly, we send representatives to accumulate knowledge from other scientists and medical personnel to constantly monitor the state of knowledge with respect to the testing, manufacture and usage of our products.

V. PRODUCT RECALLS

One of the primary reasons for interest in limiting donor exposure is to reduce the impact of the amount of material recalled. Intuitively a reduction in donor exposure should limit the amount of material recalled. This is not the case for products derived from plasma obtained by apheresis. Alpha Therapeutic Corporation maintains licenses from the FDA that allow us to fractionate either Recovered Plasma or Source Plasma into most of our products. Recovered Plasma is separated from whole blood donated from volunteer, non-remunerated donors. Source Plasma is obtained from volunteer, remunerated donors by apheresis where the cellular components are returned to the donor. Source Plasma may be collected in volumes up to 800 mL as approved by the FDA as frequently as twice per week. Recovered Plasma is generally collected in volumes of approximately 250 mL and whole blood donors may donate once every 56 days.

Alpha Therapeutic Corporation values highly the continued and frequent donations from the same individuals. This assures that we are very familiar with the health of the donor and the donor has continuously tested negative for markers of viral diseases. More than 95% of our donations are collected from repeat donors. We see most of our approximately 300,000 donors at least once per week. With these facts, a recall model for a newly emerging pathogenic agent has been constructed. This calculation is based on 2.4 million donations collected per year and a current donor exposure of 100,000 donations (similar to the currently reported values for our Venoglobulin®-S products). We assume a prevalence of one in one million donors and that the implicated donor has donated plasma once per week.

Table 12. Effect of Reduction of Manufacturing Scale on Number of Lots Recalled

	Calculation	100,000	60,000	15,000	5000
Number of donations from implicated donor		52	52	52	52
Number of donor exposures		100,000	60,000	15,000	5000
Number of donors in plasma pool	5000 liter tanks	12,000	6000	5000	5000
Number of plasma pools per year assuming same amount of plasma processed with no restrictions on manufacturing resources		200	400	480	480
Number of implicated plasma pools		52	52	52	52
Percentage of implicated plasma pools	Number of implicated pools/total number of plasma pools	26%	13%	11%	11%
Number of plasma pools combined for final container batch	donor exposure/ donors in plasma pools	8.3	10	3	1
Number of final container lots per year	number of donations/donor exposure	24	40	160	480
Number of implicated final container lots	(number of final container lots)(percentage of implicated pools)	6.2	5.2	17.6	52
Number of lots recalled	integer	7	6	18	52

With this model, if one assumes a constant yield of product from a donation of plasma we could calculate that the amount of product recalled from final container lots representing 5000 donor exposures in units would be approximately 72% of that recalled from lots with 60,000 donor exposures. The amount of product recalled is not in direct proportion to the reduction in manufacturing scale.

Upon examination of recent recalls for products derived from plasma collected from a donor with a risk factor for CJD, the number of lots associated is usually larger than the number of donors implicated. It should be noted that all recalls conducted to date have been from donors of Recovered Plasma. With a Source Plasma donor identified at risk for CJD,

[a] Because there are no combinations for the 5000 maximum donor exposure model, the number of implicated final container lots reflects the number of implicated plasma pools.

the number of lots recalled will increase depending on the frequency of the donations.

A major criticism of this model is the assumption that the same amount of plasma could be processed with unlimited manufacturing resources. In fact, this is not true. We would be limited in the number of final container lots based on equipment availability. Alpha Therapeutic Corporation has calculated the impact on capacity to produce various products at the specified manufacturing scales.

Table 13. Impact on Supply at Various Manufacturing Scales as Determined by Donor Exposure for the Active Ingredient Only

PRODUCT	15,000	5,000
Alphanate®	43%	68%
AlphaNine®-SD	50%	50%
Venoglobulin®-S	31%	41%
Albutein®	10%	20%

The calculations presented in this table demonstrate the reduction when modifying the manufacturing scale for the active ingredient only. Since Albutein® is added to both our Alphanate® and Venoglobulin®-S products, it is impossible to predict the ability to manufacture any of these products with reduced donor exposure limits beyond 60,000 without major changes in equipment and processes.

Furthermore, Alpha Therapeutic Corporation believes that for unknown agents or where we have no tests to detect potentially pathogenic agents, we should take the most conservative precautions. We recalled a radiolabeled monoclonal antibody product in clinical trials when we learned that a reagent utilized in cell culture was produced from plasma from a donor with an identified risk for CJD.

Alpha Therapeutic Corporation has backed the initiatives developed by the International Plasma Products Industry (IPPIA) to improve the effectiveness of our actions to remove products with a potential for a safety hazard from the market. IPPIA is developing a widely-publicized WEB site and a network of communications with consumer health care groups to provide information as rapidly as possible. Alpha Therapeutic Corporation has cooperated with regulatory agencies to define the appropriate actions. We have provided resources to our distributors to ease the communications to the recipients of our products. We encourage the regulation of all parts of the product distribution chain to keep accurate records including lot numbers and

amounts of lots distributed. Because our products should be administered by a physician's prescription only after accounting for the risks and benefits to an individual patient, we believe that a qualified health care agent should be available to discuss the potential health hazards with individual patients in the event of a recall or quarantine action. In the event that the recall is due to possible exposure to a pathogenic agent, the health care agent should give information on the health risks to the patient and his contacts as well as information on the type of medical support needed prevent adverse effects. Thus, we support rapid dissemination of information through knowledgeable health care providers rather than impersonal communications where affected recipients may not receive proper individualized instruction dependent on their specific medical history and needs.

VI. CONCLUSIONS

Alpha Therapeutic Corporation supports continuous improvement in measures to increase the safety margin with plasma derivatives. Our company has implemented voluntary safety measures in conjunction with industry standards to decrease safety risks with our products. Reduction in donor exposure has only a marginal effect to reduce the risk from a very rare, unknown pathogenic agent in one-time recipients only. We have implemented many more voluntary, effective safety measures with a broader impact:

- QPP viral epidemiology standards to assure that the donor population is one with low risk of viral agents such as hepatitis B, hepatitis C or HIV;
- Extended medical examination supervised by physicians to assure the continued health of our donors and preclude donors with high risk behaviors;
- Qualified Donor program with at least two donations collected with all negative viral marker tests and acceptable medical history;
- Voluntary testing of samples from each donation for liver enzyme levels as a marker for viral infection or liver disease;
- Double sample identification to prevent test result and sample mix-ups;
- Alpha currently holds donations for a minimum of 30 days and will be holding donations for a minimum of 60 days by the end of 1997 to assist in retrieval of units from a donor subsequently found to have positive viral markers or other high risk factors;
- Voluntary testing of mini-pools of 64 samples by a different test kit for HIV antibodies to confirm the absence of test errors;
- Alpha Therapeutic Corporation is a Principal Investigator on two Investigational New Drug Applications for the clinical trials of polymerase chain reaction testing of mini-pools for the absence of viral nucleic acid for HCV and HIV;
- Voluntary testing of samples of manufacturing plasma pools for the absence of hepatitis B antigen and antibodies for HIV and HCV;
- Improved viral inactivation and removal steps with the recent addition of a dry heat treatment step to solvent-detergent treated Alphanate® and a nano-filtration step to AlphaNine®-SD.

- Final product testing includes tests for the absence of hepatitis B antigen and antibodies to hepatitis C and HIV;
- Polymerase Chain Reaction testing has been implemented on samples of final container batches to test for the absence of viral nucleic acid for hepatitis A, hepatitis B, hepatitis C and HIV; and
- Voluntary on-going clinical trials to monitor the safety and effectiveness of our products.

All of these voluntary actions increase the safety of all of our plasma derivatives for all recipients.

We have committed to an upper limit of 60,000 donor exposures per final container lot. Several of our products are below that upper limit and will remain so. The further reduction of the upper limit for some of our products requires careful consideration of the consequences on product safety, efficacy and supply. Don Tankersley, a former FDA official responsible for these products, has **presented his arguments that as the manufacturing scale is decreased, there is a reduction in the safety margin**. These arguments are based on the beneficial effects of dilution and the beneficial effects of the presence of specific or fortuitous antibodies in the case of a rare, unknown pathogenic agent. Alpha Therapeutic Corporation takes seriously our responsibility to manufacture an efficacious and high quality product. The effect of dramatic reductions in manufacturing scale may affect the spectrum of antibodies present in immunoglobulin preparations administered to immunodeficient patients. We are also concerned with the inadvertent alteration of the quality of our plasma proteins by changing our processes validated through many years of safety and efficacy. The process changes required to significantly reduce the 60,000 upper limit will dramatically affect supply with hundreds of patients unable to procure their products. This supply impact will be hardest on patients in other countries where patients have only recently been able to secure a constant source of safe products.

With respect to the risk of transmission of the causative agent of Creutzfeldt-Jacob Disease, Alpha Therapeutic Corporation has added additional donor screening precautions over the mandatory screening questions. We ask the donor history related to CJD and corneal transplant since corneal transplantation has been one of the few times where CJD has been transmitted. We have implemented a donor age limit of 60 years to reduce exposure to donors with CJD. We continue to explore manufacturing steps that will remove potentially pathogenic agents such as the prion agent of CJD. We continue to explore the appropriate assays to test our processes

for removal or inactivation of such agents. Even though there has been no evidence to suggest that the causative agent of CJD is transmitted by acellular blood products or plasma derivatives, Alpha Therapeutic Corporation supports monitoring of blood and plasma derivative recipients for early recognition of transmissible agents.

With respect to recalls or other actions due to potential health hazards, Alpha Therapeutic Corporation supports rapid information exchange with recipients of our products through knowledgeable personnel able to answer questions with respect to an individual's medical history and needs. We encourage the regulation of all points in the distribution chain to maintain accurate data to rapidly effect a quarantine or recall action. We support the IPPIA initiative to develop a widely publicized WEB site for plasma derivatives and a network of communications with health care and consumer organizations.

Alpha Therapeutic Corporation has committed to cooperate with regulatory agencies to implement the best measures to reduce or eliminate safety risks. We will be ever vigilant in our quest for the safest and most efficacious products technically available.

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APPENDIX I

Federal Grants and Contracts Received by the Alpha Therapeutic Corporation Disclosure

alpha®
THERAPEUTIC CORPORATION

July 28, 1997

Congress of the United States
House of Representatives
Subcommittee on Human Resources
Christopher Shays, Chairman
Room B-372 Rayburn Building
Washington, D.C. 20515

Dear Congressman Shays:

This is the Alpha Therapeutic Corporation disclosure requested in your July 17, 1997, invitation for the upcoming July 31 hearing. This disclosure is based upon currently available and discovered information for the 1995 and 1996 fiscal years and as of June 30, 1997, of this fiscal year.

Veterans Administration - Contract no. V797P-5218N

YTD (6/30/97)	$292,710
FY 1996	$881,370
FY 1995	$606,742

Public Health Service

YTD (6/30/97)	$3,833,728
FY 1996	$4,828,641
FY 1995	$2,420,100

U.S. Department of the Army

YTD	$0
June 2, 1995, Payment	$468,083
November 15, 1994, Payment	$311,577

Very truly yours,

M. Sue Preston
Vice President
Quality and Regulatory Affairs

5555 Valley Boulevard, Los Angeles, California 90032, USA • PH: (213) 225-2221 • TELEX: 499-73

APPENDIX II

Cited References

Literature Reference 1

conjunction with the intrinsic viscosity,[21] (η), 39 ml./mL.,[6] an axial ratio of 18.3 results for the hydrated particle. This value of the axial ratio can be used in the Perrin equation to yield a friction ratio due to asymmetry of 1.92. Allowing for the fact that a hydrated particle has a frictional resistance greater than that of an anhydrated particle produces a value, 2.01, for the over-all friction ratio. Combination of this (f/f_0) value with the sedimentation constant, 185 *S.* leads to 34×10^4 for the anhydrous molecular weight. This corresponds to a rod about 270 by 14 mμ.[22]

Diffusion and sedimentation data can also be used to predict the size of the anhydrous particles. The calculation of molecular weight by this method is independent of hydration and the value, 31×10^4, previously reported still obtains. This corresponds to a rod-like particle 250 by 14 mμ for the case in which 15% by volume of water on a wet basis are associated with the virus.

The size and shape of the particle can be calculated, also, by a combination of viscosity and diffusion data. Results obtained in this manner correspond to a rod-shaped particle 250 by 13 mμ and a molecular weight of 27×10^4 when 15% of volume hydration is assumed.

(21) Intrinsic viscosity is defined as the ratio, as the volume fraction approaches zero, of specific viscosity to volume fraction.

(22) This calculation was made on the assumption that hydration increases the thickness but not the length of a virus particle.

Summary

The hydrodynamic density of tobacco mosaic virus was determined by centrifugation in bovine serum albumin and sucrose solutions of various densities. In both sets of experiments, straight lines could be used to express the dependence of sedimentation rate upon density of the medium. A hydrodynamic density of 1.13 was obtained for the experiments carried out in serum albumin solutions, and a value of 1.27 was obtained for experiments carried out in sucrose solutions. It was shown that this great discrepancy can be attributed to the effect upon the buoyancy of a virus particle produced by a disturbance of the homogeneous distribution of solute molecules in the immediate neighborhood of a virus particle. This effect depends upon the radius of the solute molecule and is therefore greater for serum albumin than for sucrose. When this effect is taken into account, the data can be interpreted to indicate that tobacco mosaic virus has an intrinsic hydration of approximately 15% by volume on a wet basis. The size and shape of the virus particle were calculated by several methods on the basis of this new hydrated model. Excellent agreement was obtained when the calculations from viscosity and sedimentation data were compared with direct measurements obtained by electron microscopy and X-ray diffraction. Calculations involving the diffusion constant gave less satisfactory agreement.

RECEIVED SEPTEMBER 10, 1948

[CONTRIBUTION FROM DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Separation of the Antibodies, Isoagglutinins, Prothrombin, Plasminogen and β_1-Lipoprotein into Subfractions of Human Plasma[1a,b]

BY J. L. ONCLEY, M. MELIN, D. A. RICHERT,[1c] J. W. CAMERON[1d] AND P. M. GROSS, JR.[1e]

The separation of the protein and lipoprotein components of human plasma into a series of fractions by the use of a five-variable system, as well as the principles involved in this fractionation, have been described in a previous paper.[2] The procedure developed depends upon the use of organic precipitants (ethanol in this case), low temperatures (0 to −8°), low ionic strengths of electrolytes (below 0.16 mole per liter), and accurate control of the pH and protein concentration. In this work the albumins were concentrated in Fraction V, fibrinogen in Fraction I, most of the α-globulins in Fraction IV-1 and IV-4. Fraction II + III contained isoagglutinins, prothrombin, plasminogen, certain lipoproteins with properties ascribed to the X-protein of plasma, as well as antibodies.[3]

The aim of this study was to devise methods

(1a) This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. From August, 1941, to July, 1946, it was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Since then it has been aided by a grant recommended by the Panel on Hematology of the National Institute of Health.

(1b) This paper is Number 73 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross, and Number XIX in the series "Preparation and Properties of Serum and Plasma Proteins" from the same laboratory.

(1c) Present address, Department of Biochemistry, Syracuse University Medical School, Syracuse, N. Y.

(1d) Present address, Citrus Experiment Station, Riverside, California.

(1e) Present address: Cobb Chemical Laboratory, University of Virginia, Charlottesville, Virginia.

(2) E. J. Cohn, L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, THIS JOURNAL, 68, 459 (1946).

(3) The importance of these methods for the concentration of antibodies was pointed out in an earlier paper of this series: E. J. Cohn, J. A. Luetscher, Jr., J. L. Oncley, S. H. Armstrong, Jr., and B. D. Davis, *ibid.*, 63, 3395 (1940).

for large scale subfractionation of as many constituents of Fraction II + III as possible into useful and stable concentrates which could then be made available for more extensive chemical and clinical work.

I. Isolation of Lipoproteins

Approximately three-quarters of the lipoproteins of plasma were concentrated in Fraction II + III. Measurements at low ionic strengths (0.005 to 0.02) indicated that both the carotenoid-bearing[4] and cholesterol-bearing proteins in this fraction had a minimum solubility at about pH 5.9. Isolated lipoprotein fractions, however, were usually isoionic near pH 5.5 (Table I).[5] The lipoprotein of Fraction II + III has the peculiar property of sedimenting in the ultracentrifuge with a rate that was very sensitive to the density of the solution. This behavior is characteristic of the so-called "X-protein" of plasma, first described by McFarlane,[6] and later by Pedersen.[7,8]

Processing of Fraction II + III, as of whole plasma, was made difficult by the presence of this lipoprotein. The total fraction could not be dried, or even frozen and thawed, without some denaturation.[10] Ammonium sulfate fractionation had yielded precipitates which either centrifuged poorly[7] or rose to the surface.[11] Separation of the lipoproteins from Fraction II + III thus became of the utmost importance.

This lipoprotein fraction, bearing the carotenoid pigment, cholesterol and most other lipids found in Fraction II + III, was extracted almost quantitatively from the residue of plasminogen, prothrombin, isoagglutinins and γ-globulins at 0.07 mole fraction (20%)[12] ethanol if the pH were about 7.6 and the ionic strength about 0.005. This ionic strength was readily achieved by resuspending Fraction II + III in a volume of this solvent equal to or twice that of the plasma from which it was obtained. The resulting precipitate (Fraction II + IIIW) contained considerable cholesterol if the extraction was carried out below pH 7, but almost none if at pH 7.6 or above, and could then be frozen and dried with little or no denaturation of the protein. The lipoprotein fraction separated was designated Fraction III-0. The advantages of this procedure, which yielded both a lipoprotein concentrate soluble in aqueous solutions and a lipid-poor precipitate, were so great that it became the first step in our subfractionation of Fraction II + III (globulin methods 8 and 9).

The extracted lipoproteins were precipitated at 0.09 mole fraction ethanol, pH 5.6 to 5.9. Because of its very low density, quantitative separation of lipoprotein from the suspension was somewhat difficult,[13] but was more complete in the presence of traces of calcium ion. The resulting lipoprotein precipitate contained a large percentage of β-globulin, and 60 to 70% of the fraction behaved as X-protein in the ultracentrifuge. Analyses revealed about 35% total lipid, about half of which was cholesterol and cholesterol esters. Materials with the properties usually ascribed to "plasmin inhibitor" and "antithrombin" were found in this fraction.[14]

Fraction III-0, like Fraction II + III, could not be dried from the frozen state without denaturation. Most of the lipoprotein was, however, precipitated as euglobulin by resuspension in a large volume of water at an ionic strength below 0.002 and at a pH between 5.4 and 5.9. The remaining protein (Fraction II + IIIW) could then be precipitated with ethanol, dried from the frozen state, and reconstituted to give a satisfactory solution.

II. Isolation of γ-Globulin Antibodies

Many antibody molecules fall into the γ-globulin class when studied by electrophoresis.[15] Proteins of this group have the most alkaline isoelectric points and the smallest electrical charges at neutral pH values of any of the major components of plasma. The γ-pseudoglobulins from horse serum[16] have an isoelectric point near pH 6.4. Human γ-globulin of this isoelectric point appears to be largely euglobulin,

(4) We are indebted to J. W. Mehl of the Department of Biochemistry, University of Southern California School of Medicine, for these observations, which were carried out at the Harvard Plasma Fractionation Laboratory during the summer of 1944.

(5) Euglobulins isoelectric in this range were reported early by W. B. Hardy (*J. Physiol.*, 33, 251 (1905)), J. Mellanby (*ibid.*, 33, 338 (1905)), H. Chick, (*Biochem. J.*, 8, 261 (1914)), and E. J. Cohn (*J. Gen. Physiol.*, 4, 667 (1922)), and more recently by A. A. Green (THIS JOURNAL, 60, 1108 (1938)). The β-globulins as a whole were reported by A. Tiselius (*Biochem. J.*, 31, 313, 1464 (1937)); (*Trans. Faraday Soc.*, 33, 524 (1937)) to be isoelectric near pH 5.1.

(6) A. S. McFarlane, *Biochem. J.*, 29, 407, 660, 1175, 1202, 1209 (1935).

(7) K. O. Pedersen, "Ultracentrifugal Studies on Serum and Serum Fractions," Almqvist and Wiksells, Boktryckeri AB, Upsala, Sweden, 1945; *J. Phys. and Colloid Chem.*, 51, 156 (1947).

(8) Solutions containing 80% of X-protein in the ultracentrifuge have been obtained in some of the euglobulin lipoprotein fractions, which were found by electrophoresis to consist largely of β_1-globulin. Further purification in the air-driven preparative ultracentrifuge has given fractions still having the sedimentation behavior of X-protein and containing as much as 97% β_1-globulin.[9]

(9) J. L. Oncley, C. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, 51, 184 (1947).

(10) L. Pillemer, during the time he was working at the Harvard Plasma Fractionation Laboratory, showed that high concentrations of certain agents, especially sucrose, seemed to render such products soluble to a considerable extent.

(11) C. S. Adair and M. E. Adair, *J. Physiol.*, 102, 17P (1943).

(12) Ethanol concentrations are expressed as mole fraction, and as per cent. by volume at 25°; see Table I, reference 2.

(13) Some additional protein could be precipitated by lowering the pH to 4.8 and slightly increasing the ethanol concentration. More albumin and α-globulin and considerably less lipid were found in this fraction.

(14) Measurements of antithrombin activity have been made by J. T. Edsall and S. G. Miller, who also have found appreciable amounts of antithrombin in Fraction IV-1.

(15) See K. Landsteiner, "The Specificity of Serological Reactions," revised edition, Harvard University Press, Cambridge, Massachusetts, 1945, Chapter IV.

(16) E. J. Cohn, T. L. McMeekin, J. L. Oncley, J. M. Newell and W. L. Hughes, Jr., THIS JOURNAL, 62, 3386 (1940).

human γ-pseudoglobulin being isoelectric near *p*H 7.4 (Table I).

TABLE I

ISOIONIC POINTS OF VARIOUS PROTEIN FRACTIONS*

Fraction	*p*H of solution of Euglobulin	*p*H of solution of Pseudoglobulin
	β-Globulin	
III-0-1,2,3:181	5.5	4.8
III-0-1,2:S302-3,4	5.5	5.4
III-0-1:183–184	5.2	4.6
III-0-3:183–184	5.6	5.7
III-0-4:S302-3,4	5.6	5.5
III-0-5:S362-3,4	5.7	5.5
III-0-5:AVL7	5.6	5.4
	γ-Globulin	
II-1:L371	6.4	7.3
II-1:LY3	6.5	7.2
II-2:L371	6.5	7.6
II-2:122	6.5	7.4
II-1,2:160A	6.3–	7.1–
II-1,2:171	6.8	7.6
II-3:SW1	6.4	7.4
II-3:42-W3F	6.2	7.0

* Determined by measuring the *p*H of the pseudoglobulin and euglobulin solutions after exhaustive dialysis, and hence representing the isoionic points of these proteins, rather than the isoelectric points. *Cf.* E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publishing Corporation, New York, N. Y., 1943, p. 446.

Solubility studies have indicated that although γ-globulin was readily precipitated by 0.09 mole fraction (25%) ethanol at neutral reaction, its solubility at *p*H 5 was considerably higher than that of albumin.[17] Sodium acetate was found to dissolve considerably more γ-globulin than sodium chloride of equal ionic strength, and both electrolytes decreased solubility with increasing ionic strength; results similar to those described for the influence of neutral salts on casein and edestin at reactions acid to their isoelectric points.[18] Great dependence of solubility upon the amount of saturating body in the system, as was earlier described for serum globulin[19,20,21] has also been observed and assumed to demonstrate the molecular heterogeneity of the γ-globulin precipitate.

The lipid-poor, readily dried, antibody-rich Fraction II + III*W* has formed an ideal starting material for the separation of γ-globulin (Fraction II) from other components (Fraction III). The γ-globulins have been extracted from these components at *p*H values of 5.8 and lower, usually 5.2. This was desirable because the γ-globulins are more soluble under these conditions than at reactions nearer neutrality, and the other components less, since they have isoelectric points in this *p*H range (5.9 to 4.8).

At any given *p*H an ethanol concentration was chosen which would permit solution of at least 3 g. of γ-globulin per liter, and in which only traces of contaminating substances were soluble.[22] Because of possible instability of various antibody molecules at acid *p*H, most efforts at isolation were carried out above *p*H 5. Reactions acid to *p*H 4.7 led to appreciably increased solubility of β-globulin, unless the ethanol concentration was greater than mole fraction 0.059 (17%). A *p*H value of about 5.2, 0.015 ionic strength, and an ethanol concentration of 0.059 mole fraction (17%) gave a very satisfactory solubility differential between the γ-globulin and the other components. A *p*H of 5.4, 0.005 ionic strength in 0.024 mole fraction ethanol (7.5%) was also satisfactory for this separation. A total Fraction II was precipitated from the supernatant by increasing the ethanol to 0.09 mole fraction and the *p*H to 7.2.

Attempts were made to separate the γ-globulins in Fraction II into subfractions. Addition of 0.05 mole/l. of sodium chloride to the γ-globulin solution at *p*H 5.2, mole fraction ethanol 0.059 (17%), and ionic strength of 0.015,[23] led to the separation of a fraction designated Fraction II-3. After Fraction II-3 had been removed, the solution was adjusted to *p*H 7.2, holding the ethanol and ionic strength constant. The precipitate formed under these conditions was called Fraction II-1. The γ-globulin remaining in solution precipitated at 0.09 mole fraction (25%) ethanol, and was called Fraction II-2.

Some physical-chemical,[9] chemical[24] and immunological[24] properties of these γ-globulin fractions have already been recorded. Clinical studies have indicated the usefulness of Fractions II-1 and II-2 in prophylaxis against measles and

(17) We are indebted to Mrs. M. H. Blanchard of this Laboratory for these studies.

(18) T. B. Osborne, THIS JOURNAL, 24, 39 (1902); T. B. Osborn and I. F. Harris, *Am. J. Physiol.*, 14, 151 (1905) (or *ibid.*, 13, 436 (1905)); K. Linderstrøm-Lang and S. Kodama, *Compt. rend. trav. lab. Carlsberg*, 16, No. 1 (1925).

(19) W. B. Hardy, *J. Physiol.*, 33, 251 (1905).

(20) J. Mellanby, *ibid.*, 33, 338 (1905).

(21) S. P. L. Sörensen, *Compt. rend. trav. lab. Carlsberg*, 15, No. 11 (1923); THIS JOURNAL, 47, 467 (1925); "Proteins," The Fleischmann Laboratories, 1925, p. 1.

(22) The γ-globulin fractions obtained by these methods have often been found to contain substances which cause a blood pressure lowering effect upon the blood pressure of certain test animals, especially cats. A number of experiments suggested that the temperature coefficient of solubility of the depressor causing substance was of a larger magnitude than that of the γ-globulin, and made it desirable to use as low a temperature as possible for the separation. L. H. Woodruff, C. A. Janeway and W. Bernberg made the earlier assays of depressor activity. More recently O. Kruyer and his associates in the Department of Pharmacology, Harvard Medical School, have investigated these effects. Some studies have also been made by E. Shorr and B. W. Zweifach of the New York Hospital.

(23) The precipitation of γ-globulin under these conditions was predicted from solubility studies,[17] and by work simultaneously carried out at the University of Wisconsin.[25] Fraction II-3 was not extracted from Fraction II + III by the earlier methods, which had been carried out in the presence of the lipoprotein now concentrated in Fraction III-0 and at higher ionic strengths.

(24) H. F. Deutsch, L. J. Gosting, R. A. Alberty and J. W. Williams, *J. Biol. Chem.*, 164, 109 (1946).

(25) J. W. Williams, M. L. Petermann, G. C. Coloyos, M. B. Goodloe, J. L. Oncley and S. H. Armstrong, Jr., *J. Clin. Invest.*, 23, 433 (1944).

(26) J. F. Rodera, *ibid.*, 23, 610 (1944).

epidemic jaundice.[27] Fraction II-3 has been shown effective in measles prophylaxis,[28] but studies in prophylaxis against epidemic jaundice have not yet been completed. Although differences among these γ-globulin fractions were observed in the above-mentioned studies, further fractionation of purified γ-globulin must be undertaken to explore more fully the possibility of separating the γ-globulin antibodies from one another.

III. Separation of Isoagglutinins, Prothrombin and Plasminogen

Solubility studies of anti-A, anti-B and anti-Rh isoagglutinins indicated a minimum solubility near 6.3, and a strong solvent action of neutral salts in water or in ethanol–water mixtures at that *p*H, and at reactions acid to the isoelectric point.[29,30] Under most conditions employed, the solubility of the fractions containing the isoagglutinins was considerably lower than that of other γ-globulins. Quantitative measurements indicate, however, that less than 1% of even the most highly purified fractions were agglutinating antibody.[31]

Highly purified human prothrombin has thus far not been prepared. However, as Mellanby's important observations suggested, it appears to be a euglobulin with a minimum solubility near *p*H 4.8, dissolved by most electrolytes in aqueous solutions when the ionic strength was 0.08 mole per liter or greater.[32,33] Most investigators have reported inactivation of prothrombin at reactions acid to *p*H 4.8 and alkaline to *p*H 10. If it is assumed that human prothrombin has the same activity as the bovine prothrombin highly purified by Seegers and reported to be 1500 u./mg.,[33] then fractions with activities of less than 30 u./mg., routinely prepared in the course of this work, contained less than 2% prothrombin.[34]

Although there was no need to purify this component further, when it is prepared to yield human thrombin for clinical purposes[34] the fractions containing prothrombin which have been obtained must be considered only as starting material for further chemical investigations.

Plasma, or a fraction of plasma, has been rendered proteolytic by the use of certain reagents[36]; the activated enzyme termed plasmin (fibrinolysin), and the inactive enzyme (or precursor), plasminogen.[37] Under most conditions investigated, plasminogen precipitated with prothrombin. The observation that plasminogen was adsorbed by fibrin made possible its separation from prothrombin.

The separation of the isoagglutinins from prothrombin and plasminogen was made possible by utilizing the different isoelectric points of these materials. At *p*H 5.4 there were large differences in solubility, and the solubilities of the two components to be separated can be adjusted to suitable values by the variation of the ionic strength of an aqueous solution. Since it has been observed that prothrombin was considerably less soluble in sodium acetate buffers[38] than in sodium chloride solution with *p*H adjusted to the same value, and since a specific effect of the acetate ion upon the solubility of the isoagglutinins was not noted, it was found advantageous to use acetate buffers for this step. This separation was effected by completely dissolving Fraction III in a small volume of acetate buffer of about 0.2 ionic strength, and then precipitating the prothrombin and plasminogen (Fraction III-2,3) together with most of the remaining fibrinogen and a part of the other proteins present by diluting this solution

(27) C. W. Ordman, C. G. Jennings, Jr., and C. A. Janeway, *J. Clin. Invest.*, **23**, 541 (1944); C. A. Janeway, *Bull. N. Y. Acad. Med.*, **21**, 202 (1945); *J. Am. Med. Assoc.*, **126**, 674 (1944); M. Greenberg, S. Frant and D. D. Rutstein, *ibid.*, **126**, 944 (1944); J. Stokes, Jr., and J. R. Neefe, *ibid.*, **127**, 144 (1945).

(28) C. A. Janeway, personal communication.

(29) The presence of anti-A and anti-B isoagglutinins in Fraction II + III was noted by W. C. Boyd, and reported in some detail by J. F. Enders, *J. Clin. Invest.*, **23**, 510 (1944), (*cf.* table on p. 515, compiled by W. C. Boyd), and by L. Pillemer, J. L. Oncley, M. Melin, J. Elliott and M. C. Hutchinson, *J. Clin. Invest.*, **23**, 550 (1944).

(30) Studies of the anti-Rh isoagglutinins have been carried out in collaboration with L. K. Diamond, Department of Pediatrics, Children's Hospital, Boston, Mass.

(31) E. A. Kabat, private communication. See also E. A. Kabat and A. B. Berer, *J. Exptl. Med.*, **82**, 207 (1945).

(32) For a discussion of the earlier literature see J. T. Edsall, R. M. Ferry and S. H. Armstrong, Jr., *J. Clin. Invest.*, **23**, 557 (1944).

(33) A recent survey of the properties of prothrombin has been made by W. H. Seegers, B. C. Loomis and J. M. Vandenbelt, *Proc. Soc. Exptl. Biol. and Med.*, **56**, 70 (1944); and *Arch. Biochim.*, **6**, 85 (1945).

(34) The assay of thrombin and prothrombin was briefly described by Edsall, Ferry and Armstrong.[32] More recently, it has been defined in terms of a standard thrombin reference preparation, whose activity is fixed by definition. This new thrombin unit is identical within the limits of experimental error with the thrombin unit described by Seegers,[33] and has been officially adopted by the National Institute of Health (defined in detail in Minimum Requirements for Dried Thrombin, National Institute of Health, August 28, 1945). See the reports on the determination of prothrombin in "Blood Clotting and Allied Problems," Josiah Macy, Jr., Foundation New York, 1948.

(35) E. A. Bering, Jr., *J. Clin. Invest.*, **23**, 586 (1944); O. T. Bailey and F. D. Ingraham, *ibid.*, **23**, 591 (1944); F. D. Ingraham and O. T. Bailey, *J. Neurosurg.*, **1**, 23 (1944); F. D. Ingraham, O. T. Bailey and F. E. Nulsen, *ibid.*, **1**, 171 (1944); O. T. Bailey, F. D. Ingraham, O. Swenson, J. J. Lowrey and E. A. Bering, Jr., *Surgery*, **18**, 347 (1945).

(36) In Fraction III-3, the separation of which is described below, D. A. Richert found a slow spontaneous activation of plasminogen to plasmin, even in sterile solution, without the addition of chloroform, streptokinase, or any other activator. The final concentration of plasmin obtained in this way was about one-third of the maximum obtainable on complete activation with streptokinase. Chloroform treatment of Fraction III-3 did not give any increase in the rate or in the final amount of activation, as compared with the control sample without added activator. This is perhaps explainable by the fact that the lipids present in Fraction II + III were previously separated in Fraction III-O. The function of the chloroform in whole plasma or crude plasma fractions may well be due to its extraction of fat soluble inhibitors which normally prevent the spontaneous conversion of plasminogen to plasmin. For further discussion of the proteolytic enzyme system of plasma, see J. T. Edsall, "Advances in Protein Chemistry," **3**, 449–61 (1947).

(37) In order to describe the protease system the revised terminology suggested by L. R. Christensen and C. M. MacLeod, *J. Gen. Physiol.*, **28**, 559 (1945), has been followed.

(38) These observations were made by L. Pillemer, D. J. Mulford, J. T. Edsall, S. G. Miller and D. A. Richert.

with water to an ionic strength of about 0.08.[39]

The recovery of isoagglutinin in the supernatant solution from Fraction III-2,3 was accomplished by raising the *p*H to 6.3 (minimum solubility of the isoagglutinins), and adding a small amount of ethanol. The recovery of the isoagglutinins was quite satisfactory, and the precipitate so obtained (Fraction III-1) was dried from the frozen state without deterioration.

Prothrombin and plasminogen were separated by dissolving Fraction III-2,3 in about 0.1 ionic strength sodium chloride and sufficient buffer to bring the *p*H to about 6.9. Under proper conditions a fibrin clot[40] formed and was removed by centrifugation. This clot lysed in a few hours at room temperature, or after standing overnight in the cold room, and contained a large part of the plasminogen.[41]

The supernatant solution from the clot contained most of the prothrombin, which could be reprecipitated by lowering the *p*H and adding a small amount of ethanol, or could be dried from the frozen state. Prothrombin was not stable indefinitely, but could be readily converted to thrombin by suitable agents.[42,43]

IV. Procedures for the Subfractionation of Fraction II + III[43]

Studies of the subfractionation of Fraction II + III involving isoelectric precipitation at various ionic strengths, but without the use of ethanol, yielded fairly active prothrombin fractions, but failed to yield concentrated antibody-containing fractions which were free of lipid. Various methods involving precipitation with ethanol, with careful control of the *p*H, ionic strength and temperature, were then introduced. Only the last methods have led to the separation of all protein components in conditions approximating the native state. For this reason it has not seemed desirable to describe in detail earlier attempts at complete fractionation. Since they may be useful for specific purposes, however, they have been briefly outlined.[44] Although developed for the fractionation of normal human plasma, they have also been applied with some success to certain types of convalescent human plasma and to the plasma of certain animals. Modifications of these methods may lead to more satisfactory separations of the comparable proteins of different species, because of their somewhat different concentrations and solubilities.

Method 1 for preparing the total Fraction II + III for immunological studies was introduced in 1942. The precipitated Fraction II + III, removed and stored at a temperature of −5°, contained approximately 35% protein by weight, 0.002 mole fraction (18%) ethanol, and 0.06 ionic strength of salts (largely sodium chloride with some citrate and bicarbonate). This material was suspended in about an equal volume of 0.1 *M* sodium chloride solution previously chilled to 0°, and dialyzed against 0.1 *M* sodium chloride at 0° for two or three days with frequent changes of dialysate to remove the ethanol present in the precipitated material. During dialysis a clot of fibrin usually formed and was removed by filtration and centrifugation. The precipitate was then washed with cold 0.5 *M* sodium chloride solution, recentrifuged, and the supernatant solution was added to the dialyzed material. The resulting protein solution, containing only traces of ethanol, was adjusted to 0.15 *M* sodium chloride, approximately 15% of protein by dry weight. It could be sterilized by filtration through Seitz type filter pads. Thrombin was largely removed during clotting, and prothrombin was removed by the filtration.

Method 2 made available γ-globulin and thrombin. Fraction II + III was suspended in cold sodium chloride solution, allowed to clot, and clarified. Prothrombin (Fraction III-2) was removed by adjusting to *p*H 4.7–5.3 and an ionic strength of about 0.03. The remaining α- and β-globulin (Fraction III-1) was removed at 0° by adjusting the *p*H to 5.7, the ionic strength to 0.015 or 0.03 and the ethanol concentration to about 0.051 mole fraction (15%). The volume used was about twice that of the original plasma. The γ-globulin (Fraction II) was then precipitated by increasing the ethanol concentration to mole fraction 0.091 (25%), which precipitated Fraction II-2. By then adjusting the *p*H to 7.0, Fraction II-1 was precipitated.

Method 3 involved suspension of Fraction II + III paste, clotting of fibrinogen, and removal of thrombin by methods similar to those used in Method 2. Fraction III-1 was then removed at more acid *p*H (5.2 to 4.5), lower volume (approximately equal to the volume of original plasma), and ethanol concentrations from 0.04 to 0.07 mole fraction (12 to 20%) (Table II). The total γ-globulin was usually precipitated in one fraction, but in some cases Fractions II-1 and II-2 were removed separately.

Method 4 involved suspension of Fraction II + III in a volume of water twice that of the original plasma at *p*H 5.9, 0.005 ionic strength, 0°. The resulting euglobulin precipitate represented about half the total protein, and contained considerable γ-globulin. The supernatant solution, precipitated by bringing the ethanol concentration to 0.1 mole fraction (30%), contained large amounts of γ-globulin and lipoprotein.

Method 5 was developed to make isoagglutinins available in good yield. Suspension, clotting of fibrinogen, and removal of prothrombin were accomplished as in Methods 2 and 3, except that Fraction III-2 was precipitated at a slightly lower ionic strength (0.06). Fraction III-1, containing β-globulin, lipoprotein and isoagglutinin, was precipitated in a volume 1.8 times that of the plasma represented, at *p*H 6.3, 0.015 ionic strength, 0.028 mole fraction (8%) ethanol, 0°. The ethanol was removed by suspending the precipitate in about 20 volumes of water at 0°, *p*H 6.3, and ionic strength less than 0.001. The resulting lipoprotein-rich precipitate could not be dried without denaturation, but the paste could be suspended in 0.15 *M* sodium chloride and adjusted to *p*H 7.2. The γ-globulin was precipitated as in Method 3.

(39) These conditions are very nearly those used in the original Mellanby procedure.[5a] A similar procedure, using somewhat lower ionic strengths, was used in the isolation of the C'1 component of complement from guinea pig serum, L. Pillemer, R. R. Ecker, J. L. Oncley and E. J. Cohn, *J. Exptl. Med.*, 74, 297 (1941).

(40) If sufficient thrombin had been formed by converting agents in the plasma, spontaneous clotting occurred. If insufficient thrombin were present, a small amount was added at this point.

(41) The products of the lysis of the fibrin clot have been studied by W. H. Seegers, M. L. Nieft and J. M. Vandenbelt, *Arch. Biochem.*, 7, 15 (1945), who found two main components called α- and β-fibrin. The α-fibrin, *pI* = 5.5, has an electrophoretic behavior similar to fibrinogen; the β-fibrin, *pI* = 4.2, is somewhat more like α-globulin or albumin.

(42) See L. E. Strong, Encyclopedia of Chemical Technology, Vol. II, p. 571–572. The Interscience Encyclopedia, Inc., New York, N. Y., 1948.

(43) The materials and method used for these subfractionations are completely described in ref. 2.

(44) Detailed procedures for carrying out most of these methods have been published in the Bulletin of the Blood Substitutes Committee, Committee on Medical Research, Office of Scientific Research and Development. They will be made available upon request.

TABLE II

CONDITIONS FOR THE SEPARATION OF FRACTION III-1 FROM γ-GLOBULIN

Method	pH	Ionic strength, moles/L.	Ethanol concn. Mole fraction	Vol. % (25°)	Temperature, °C.	Volume compared to plasma
2	5.7	0.015–0.03	0.051	15	0	2.0
3A	5.1	.040	.048	14	−3	1.0
3B[a]	5.2	.059	.040	12	−3	1.0
3C	5.2	.036	.051	15	−5	0.9
3D	5.2	.036	.059	17	−3	.9
3E	5.2	.036	.059	17	−5	.9
3F	5.2	.036	.059	17	−6	.9
3G	4.8	.036	.070	20	−7	.9
3H[a]	4.6	.036	.070	20	−7	.9
5	6.3	.015	.026	8	0	1.8
8	5.4	.005	.024	7.5	−2	0.7
9	5.2	.015	.059	17	−6	0.7

[a] The γ-globulin fraction obtained from these conditions was found to contain considerable β-globulin as impurity.

Method 6 was used to obtain a satisfactory isoagglutinin fraction when other fractions were not desired. Fraction II + III was suspended and taken to pH 6.3, 0.01 ionic strength, 0.024 mole fraction (7.5%) ethanol, 0°, and a volume about 0.6 times that of the original plasma. Isoagglutinins were precipitated along with lipoprotein. The resuspended precipitate was adjusted to pH 7.2 in water at 0°, 0.005 ionic strength, and a volume 0.7 times that of plasma. Much of the lipoprotein and γ-globulin were soluble under these conditions, but the isoagglutinins remained in the precipitate. This precipitate was dissolved in 0.1 *M* sodium chloride, and thrombin was added to remove fibrinogen as a fibrin clot.

Method 7 recovered only the isoagglutinin fraction.[7] Fraction II + III was suspended at 0° or 25° in a volume of water eight times that of the plasma, pH 6.3, and an ionic strength of 0.005. The water was saturated with toluene. The isoagglutinin fraction settled in about twenty-four hours. Resuspension under the same conditions was sometimes desirable. Fibrinogen and most of the γ-globulin and lipoprotein were removed by this procedure.

Method 8 was similar to method 9, except that Fraction III was removed at pH 5.4, 0.005 ionic strength, 0.024 mole fraction (7.5%) ethanol, −2°, and a volume 0.7 times the volume of plasma represented.

Method 9 evolved from the combination of method 8 with the modification of method 3 to a lower ionic strength developed at the University of Wisconsin[24] for the separation of Fraction III from γ-globulin. This method is given in detail in the remainder of this section.

Precipitate II + III from standard preparations of normal serum albumin (human) is used as the starting material for this preparation.[15] Each kilogram of this precipitate is suspended in 2 kg. of water containing ice (about one-quarter of the water should be frozen, in the form of very fine ice crystals). When the suspension is fairly uniform, add 3 kg. of water (cooled to 0°) to which 112 cc. of 0.5 *M* disodium phosphate (pH 9.2) has been added.[46] The suspension should be stirred slowly and kept at a temperature of 0° until all lumps are dissolved and a nearly complete solution is obtained. After the suspension is complete, add it to 20 kg. of water (cooled to 0°), and stir slowly at 0° for thirty to sixty minutes. The pH of this suspension[47] should be 7.6 ± 0.2. Then bring to 20% ethanol by adding 15 liters of 53.3% ethanol keeping the temperature as low as possible until −5° is reached. This suspension should stand at −5° with a slow stirring for several hours before centrifuging. Centrifuge at a rate of about 30 liters per hour.

Precipitate III-O is precipitated from the supernatant from precipitate II + IIIW by bringing the pH to about 5.7, with pH 4 calcium acetate buffer,[48] and the ethanol concentration to 25%. This suspension should be allowed to stand for about forty-eight hours at −5° before centrifugation, and then centrifuged at a rate of about 20 liters per hour. The supernatant solution still contains appreciable protein, a large part of which is precipitated by adjusting the pH to 4.8, and the ethanol concentration to 35%.

Precipitate II + IIIW is resuspended in acetate buffer[49] and taken to pH 5.2, 0.015 ionic strength and 17% ethanol. This can be done by suspending each kilogram of precipitate II + IIIW in 2 kilograms of water and ice, as before, and then adding 2 kg. of cold water to which 0.35 mole of sodium acetate has been added. When this suspension is complete, add sufficient pH 4 acetate buffer,[51] diluted with one liter of cold water per kilogram of precipitate II + IIIW, to lower the pH of the suspension to 5.0 ± 0.1 and stir for several hours. Then add 13.5 liters of cold water and then 8.60 liters of 53.3% ethanol per kilogram of precipitate II + IIIW, raising the ethanol concentration to 17%. The temperature should be lowered during the addition of the ethanol, keeping the suspension near the freezing point until it is cooled to −6°. The precipitate is re-

(45) This method can also be applied to precipitate II + III or I + II + III from special lots of plasma or serum, such as convalescent serum or plasma, or to various similar fractions obtained by these methods. Occasionally, where materials have been stored in the frozen or dry state, the original suspension will yield a considerable quantity of insoluble material consisting of denatured protein and lipid. In these cases, material can be centrifuged and clarified with filtration through Seitz-type pads in a solution of 1 or 2 per cent. protein and 0.15 *M* sodium chloride at pH about 7. The clarified solution can then be taken to −5°, pH 6.8, 25% ethanol, ionic strength 0.1 just as plasma would be treated (but omitting the removal of Fraction I), and the precipitate corresponding to the usual Fraction II + III can then be used in this preparation.

(46) A sodium glycinate buffer solution was also used for this pH adjustment. See ref. 53.

(47) Because of the low ionic strength of this suspension, the pH value obtained depends considerably upon the method of reading in the glass electrode. We have used the value obtained by dilution of the suspension just before the alcohol addition with an equal volume of 0.02 *M* sodium chloride solution. Dilution with an equal volume of 0.15 *M* sodium chloride solution will give a pH nearer 7.2

(48) The pH 4 calcium acetate buffer is that used in the fractionation of plasma except that the sodium acetate is replaced by calcium acetate. It consists of 4.0 *M* acetic acid and 0.4 *M* calcium acetate, and is diluted before use.

(49) When we are dealing with plasma collected so that it contains a high titer of the various anti-Rh isoagglutinins[24] it is often advantageous to process the Fraction II + IIIW by a modification of Method 7. The procedure which has been used follows:

Precipitate II + IIIW is resuspended in water and taken to pH 6.3, 0.005 ionic strength, using acetate buffer. This can be done by suspending each kilogram of precipitate II + IIIW in two liters of cold water and ice. When this suspension is complete, pour it with good stirring into 90 liters of 0.005 ionic strength pH 6.3 acetate buffer per liter of starting II + IIIW paste. This resulting suspension may be held at either 0° or 25°. It is usually carried out at 0°, but where there is a large amount of fibrinogen present in the precipitate II + IIIW the 25° extraction procedure is advantageous. When the extraction is carried out at room temperature, the buffer is saturated with toluene as a bacteriostatic agent (approximately 0.6 cc. per liter). The dilute suspension is then stirred for about an hour, and then allowed to settle overnight. The precipitate is suspended in a small amount of pH 6.3 buffer of 0.005 ionic strength (sodium acetate), frozen and dried in vacuum.

This method can also be applied to precipitate III as obtained from method 9 for the subfractionation of Fraction II + III.

The use of this material as a reagent for blood typing has been discussed in an earlier paper.[24]

(50) J. L. Oncley, M. Melin, J. W. Cameron, D. A. Richert and L. K. Diamond, *Ann. N. Y. Acad. Sci.*, 47, 899 (1946).

(51) The pH 4.0 sodium acetate buffer is that used in the fractionation of plasma.[5] It consists of 4.0 *M* acetic acid and 0.8 *M* sodium acetate.

moved by centrifugation at a rate of about 30 liters per hour and at a temperature of $-8.0 \pm 0.5°$. The effluent from this centrifugation should then be clarified by filtration[52] at a temperature of $-8.0 \pm 0.5°$.

Each kilogram of precipitate III is suspended in 2 kg. of water and ice, and then diluted with 2 kg. of 0.5 ionic strength pH 5.4 acetate buffer cooled to 0°. The suspension is stirred until uniform, and then diluted with 7.5 kg. of 0° water. This suspension should be at pH 5.4, ionic strength 0.08, and 0 to +1°, and should be stirred for several hours. The precipitate is removed by centrifugation at a relatively slow rate of speed and at 0 to +1°.

Precipitate III-2,3 should be suspended as soon as possible in 1 liter of 0° water per kilogram of precipitate and the pH adjusted to 6.8 to 7.0 with sodium glycinate buffer.[53] Then add 1 liter of 0° water containing enough sodium chloride to give a total ionic strength of 0.1. After one to two hours of stirring, test the solution for preformed thrombin and remove a sample to see if the fibrinogen has clotted. To do this, centrifuge a small sample and then add thrombin to the supernatant solution to see whether more fibrin is formed. If all the fibrinogen has clotted, do not add additional thrombin. If there is fibrinogen still left in solution, then add enough thrombin to give a total amount (including that already present) of 10,000 units per kilogram of Fraction III-2,3. Stir this suspension for an additional hour and centrifuge at 0° for about an hour, or at a very slow rate if continuous centrifugation is required. The supernatant containing Fraction III-2 can either be dried directly if prothrombin is desired, or immediately converted to thrombin before drying. To accomplish the conversion of prothrombin to thrombin, calcium chloride and thromboplastin are added, according to the directions given elsewhere,[43] and the temperature is raised to 20–25°. This solution is then clarified, sterilized and dried.

Precipitate III-3 should be suspended[54] in sodium chloride and bicarbonate solution to give an ionic strength of 0.15 and a pH of 7.1 ± 0.1, and allowed to stand at 0° until all of the fibrin has been lysed. After clarification and sterilization, this material can be dried from the frozen state.

Each kilogram of the supernatant from precipitate III-2 is adjusted to pH 6.3 by the addition of sodium bicarbonate (approximately 20 cc. of 1 *M* sodium bicarbonate should be required), and then the ethanol is adjusted to 15% by the addition of an equal volume of 30% ethanol, keeping the temperature at or near the freezing point until lowered to −5°, where it should be maintained. The precipitate can then be removed by centrifugation at −5°, and at a speed of about 80 liters per hour.

Precipitate III-1 can be dried from the frozen state, and reconstituted to about 5% protein in isotonic saline at pH 7 for a blood grouping solution if the pool were group specific and of sufficiently high titer.[55,56]

Each kilogram of filtrate from precipitate III is taken to about 0.06 ionic strength by the addition of 50 millimoles of sodium chloride, holding the pH at 5.2, the ethanol concentration at 17%, and the temperature at −6°.

Precipitate II-3 can be removed by centrifugation of the above suspension at a rate of about 50 liters per hour and at a temperature of −5°. It may be dried from the frozen state[56] preferably without the addition of sodium chloride or glycine.

Each kilogram of filtrate from precipitate II-3 is taken to pH 7.4 ± 0.2 by addition of about 15 millimoles of sodium bicarbonate, and to 20–25% ethanol by the addition of 40–114 cc. of 95% ethanol. The temperature is maintained at −5°.

Precipitate II-1,2[57] can be removed by centrifugation of the above suspension at a rate of about 50 liters per hour, and at a temperature of −5°. It may be dried from the frozen state preferably without the addition of sodium chloride or glycine.

Precipitate II.—A total Fraction II, rather than the Fractions II-1,2 and II-3, can be prepared from the filtered effluent from precipitate III by increasing the pH to 7.4 ± 0.2 with sodium bicarbonate solution, and the ethanol concentration to 25% with 95% ethanol. The temperature should be maintained at −5°. The precipitate may be removed by centrifugation at the same rate as recommended for Fractions II-1,2 and II-3.

V. Distribution of Proteins in the II + III Subfractions

Analyses of the various steps in the fractionation procedure have been carried out by the same methods already reported in a previous study of this series (2). The results obtained during the fractionation of a large lot of Fraction II + III prepared by method 6 for the fractionation of citrated plasma (2) are recorded in Table III. Nitrogen determinations and volume measurements were made on all suspensions and supernatant solutions obtained during subfractionation, and of all resuspended precipitates. Values for the protein in the precipitates are calculated from the analyses of the resuspended precipitates, and the protein lost in centrifugation or filtration, and the protein remaining in the supernatant solutions at the end of the process were calculated from the analyses of the suspensions or supernatant solutions. These results are tabulated as the percentage of total protein nitrogen in the fraction referred to the protein nitrogen in the Fraction II + III used for subfractionation. To convert these values to the amount of protein by nitrogen (N × 6.25) which would have been obtained from a liter of plasma, we have used the value of 15.1 g. of Fraction II + III per liter of plasma previously reported.[58] The nitrogen factors estimated from nitrogen and dry weight determinations of each resuspended precipitate are also recorded in Table IV. Using these nitrogen factors, instead of 6.25 as used in Table III, we obtain the values recorded in the last column of Table

(52) We have used Seitz S-1 pads and 0.5% 505 filter aid and sometimes K-5 pads and 1% Hyflow filter aid for this filtration. The filter pad should be washed with 0.30 *M* sodium chloride and then with 0.01 *M* acetic acid, and finally with 0.05 ionic strength pH 5.2 acetate buffer in 17% ethanol. The pH of the final washings should be checked to see that it is 5.2 after filtration (this can be easily done with a spot-plate and suitable indicator solution). This washing procedure is essential because the low ionic strength of the solution and changes in pH at this point can substantially lower the recovery of γ-globulin.

(53) Sodium glycinate buffer is prepared by half-neutralizing a glycine solution with sodium hydroxide. The solution we have used contains 1 mole of glycine and 0.5 mole sodium hydroxide per liter. It has a pH of about 9.5 and has the advantage of causing less of a drift in pH upon aeration than is observed when sodium bicarbonate is used to increase the alkalinity.

(54) The suspension of this material is conveniently carried out using a Waring blendor to break up the fibrin clot.

(55) M. Melin, *J. Clin. Invest.*, 24, 602 (1945).

(56) We have found it helpful to suspend precipitate II-3 and II-1,2 in 1.5 volumes of water–ice mixture (1.2 of water frozen). If this mixture is kept cold during the suspension, most of the globulin remains undissolved, and does not "lump up" upon freezing.

(57) Fraction II-1,2 represents the more soluble part of the γ-globulin and is taken off at conditions used for preparation of Fraction II by earlier methods. Such material has been used clinically in both measles and epidemic jaundice. This fraction should be dissolved to make solutions containing 16.5 g. of γ-globulin per 100 cc. and sterilized by Seitz filtration.

(58) See Ref. 2, Table VII.

TABLE III

DISTRIBUTION OF PROTEINS IN SUBFRACTIONS OF FRACTION II + III

Experiment	Per cent. of total protein nitrogen in fraction 1	2	3	4	Mean	C, N × 6.25 per liter of plasma
II + III	(100)	(100)	(100)	(100)	(100)	(15.1)
II + IIIW	66.0	65.0	62.5	61.5	63.7	9.6
Cent. loss	0.7	1.0	0.9	1.5	1.0	
III-0	24.4	26.2	22.7	24.3	24.4	3.7
Cent. loss	0.6	0.8	1.8	0.7	1.0	
Supernatant	8.3	7.0	12.1	12.0	9.9	
III	.... 26.8	....	.... 24.2	....	25.5	3.9
Cent. loss	.... 1.2	....	.... 2.0	....	1.6	
Filt. loss	.... 2.8	....	.... 2.0	....	2.4	
II-3	.... 20.9	....	.... 15.9	....	18.9	2.9
Cent. loss	.... 0.2	....	.... 0.2	....	0.2	
II-1,2	.... 13.2	....	.... 15.5	....	14.4	2.2
Cent. loss	.... 0.1	....	.... 0.1	....	0.1	
Supernatant	.... 0.4	....	.... 0.9	....	0.6	
III-1	.... 11.9	....	.... 11.2	....	11.6	1.8
Cent. loss	.... 0.6	....	.... 0.5	....	0.6	
Supernatant	.... 1.3	....	.... 0.7	....	1.0	
III-2	.... 8.8	....	.... 7.2	....	8.0	1.2
Cent. loss	.... 0.9	....	.... 0.7	....	0.8	
III-3	.... 2.7	....	.... 2.6	....	2.7	0.4
Cent. loss	.... 0.6	....	.... 1.3	....	0.9	

V(b) for the grams of protein per liter of plasma recovered in fractions.

We have recorded the electrophoretic analyses of the various II + III sub-fractions in Table V(a). In some cases the separations between these components is incomplete, especially in the case of the β_1-globulin and the β_2-globulin + fibrinogen components. The protein distributions in Tables V(b) and V(c) have been corrected for the nitrogen factor, but not for the refractive index increment of the components. Recent papers in this series[59] have indicated that these refractive index corrections would be small, and they can properly be neglected at this time. The unusually high mobilities assigned to the β_1 component in the lipid-rich fractions has also been discussed.[59]

TABLE IV

ESTIMATES OF NITROGEN AND CHOLESTEROL IN SUBFRACTIONS OF FRACTION II + III OF HUMAN PLASMA

Fraction	Grams protein/gram nitrogen (1)	(2)	(3)	(4)	Mean	Wt. per cent. Nitrogen	Wt. per cent. Cholesterol
II + III	7.9	7.8	7.8	7.8	7.8	12.8	6.0
II + IIIW	—6.8—		6.7	6.7	6.7	14.9	1.8
III-0	10.5	10.4	9.9	9.8	10.1	9.9	12.
II-1,2	——6.2[a]——				6.2[b]	16.0	<0.06
II-3	——6.2[a]——				6.2[b]	16.0	<0.06
III	—7.1—		—7.0—		7.0	14.3	3.5
III-1	—7.3—		—7.2—		7.3	13.8	3.7
III-2	—6.8—		—6.6—		6.7	15.0	3.0
III-3	—6.5—		—6.7—		6.6	15.2	1.2

[a] After filtration of combined 16.5% γ-globulin solution. [b] Also value of E. Brand, B. Kassell and L. J. Saidel, *J. Clin. Invest.*, 23, 437 (1944).

(59) S. H. Armstrong, Jr., M. J. E. Budka and K. C. Morrison, THIS JOURNAL, 69, 416 (1947); S. H. Armstrong, Jr., M. J. E. Budka, K. C. Morrison and M. Hasson, *ibid.*, 69, 1747 (1947).

TABLE V

DISTRIBUTION OF PLASMA PROTEINS INTO II + III SUBFRACTIONS BY METHOD 9

Fraction	A	α	β_1	$\beta_1 + \beta_2$	γ	Total
	(a) Per cent. of fraction					
II + III	4	6	35	18	37	100
III-0	6	5	68	16	5	100
II + IIIW	2	8	15	19	56	100
II-1,2	0	0	0	2	98	100
II-3	0	0	0	4	96	100
III	3	15	33	34	15	100
III-1	2	18	16	45	14	100
III-2	1	20	65	12	2	100
III-3	3	20[a]	39	37[b]	2	100
	(b) Grams per liter of plasma recovered in fractions					
II + III	0.7	1.1	6.5	3.4	6.9	18.6
III-0	0.3	0.3	4.1	1.0	0.3	6.0
II + IIIW	0.2	0.8	1.6	1.9	5.8	10.3
II-1,2	0	0	0	0	2.2	2.2
II-3	0	0	0	0.1	2.8	2.9
III	0.1	0.7	1.5	1.5	0.6	4.4
III-1	0	0.4	0.3	1.0	0.3	2.1
III-2	0	0.3	0.8	0.2	0	1.3
III-3	0	0.1	0.1	0.2	0	0.4
	(c) Grams per liter of plasma estimated in plasma					
II + III	0.8	1.1	6.6	3.5	7.0	19.0
II	0	0	0	0.1	5.4	5.5
III-1	0.1	0.5	0.4	1.2	0.4	2.6
III-2	0	0.4	1.0	0.2	0.1	1.7
III-3	0	0.2	0.3	0.3	0	0.8
III-0	0.5	0.5	5.7	1.3	0.4	8.4
Total	0.6	1.6	7.4	3.1	6.3	19.0

[a] Including β-fibrin.[61] [b] Including α-fibrin.[61]

In order to estimate the composition of plasma from these data, we have also calculated the amounts of each of these subfractions present in a liter of plasma (Table V(c)), using the data in Table III to estimate the losses. Although the lost material probably does not have the same electrophoretic composition as the isolated subfractions, we have calculated the distribution of proteins using this assumption, and the total found in the corrected subfractions is near to that observed for the Fraction II + III.

The distribution of the lipoprotein components has been followed by cholesterol determinations in most of these fractions, as recorded in Table IV. These values, when combined with the yield of the fractions from Table V(c), indicate that about 85% of the cholesterol of II + III, or about two-thirds of the cholesterol of plasma, is concentrated into Fraction III-0. The purified γ-globulin fractions (II-1,2 and II-3) contain less than one-fourth molecule of cholesterol per molecule of γ-globulin.

TABLE VI

DISTRIBUTION OF CERTAIN ANTIBODIES INTO II + III SUBFRACTIONS BY METHOD 9

Fraction	Weight of fraction	Typhoid agglutinin O	Typhoid agglutinin H	Influenza A Hirst test	Influenza A mouse Prot.	Diphtheria antitoxin	Streptococcal Antitoxin
		(a) Preparation 184 (Ratio to IIG141)					
II-1,2		1.1	1.5	0.6	0.7	0.8	1.0
II-3		4.6	1.3	1.2	.9	.6	0.9
III-1		40	0.9	0.7	.1	.3	...
III-2,3		20	0.7	.2	.1	.1	...
III-0		...	...	.2	.1	.1	...
Plasma		4.6	0.3	.3	.2	.1	...
		(b) Preparation S362 (Ratio to IIG141)					
II-1,2		1.0	1.0	1.0	1.0	1.2	1.0
II-3		3.8	1.4	1.0	0.7	1.3	0.8
III-1		41	3.8	0.7	.5	0.5	...
III-2		10	0.6	0.3	.1	.1	...
III-0		0.7	0.2	0.2	.1	.1	...
II + III		10	.1.5	1.2	.3	.7	...
		(c) Preparation 184 (per cent. of plasma)					
II-1,2	3.8	1	18	7	16	20	
II-3	4.6	5	18	10	22	25	
III-1	4.0	36	12	9	3	6	
III-2,3	3.8	17	8	2	2	1	
III-0	12.7	...	...	6	6	10	
Total	28.9	59	56	43	48	64	
		(d) Preparation S362 (per cent. of II + III)					
II-1,2	13	1	9	10	39	21	
II-3	16	6	15	13	34	29	
III-1	14	59	35	7	23	10	
III-2	9	9	4	2	2	1	
III-3	4	...	..	..	...	..	
III-0	44	2	2	8	10	7	
Total	100	77	65	40	108	68	

VII. Distribution of Antibodies and Physiologically Active Components

Studies of the antibody content of the various fractions have been reported by Enders.[16] More extensive studies are now available.[60] Nearly all antibodies which have been studied are found concentrated into Fractions II-1,2, II-3 or III-1, and Fractions I, III-0, III-2, III-3, IV-1, IV-4 and V are found to contain only traces of antibody. Table VI gives the results obtained on two preparations which were carefully studied. The results are first compared with a reference solution containing the same concentration of protein Table VI (a) and (b), and then with the plasma (or Fraction II + III for preparation S362) from which it was fractionated. The totals recorded in Table VI (c) and (d) represent the amount of antibody accounted for in the various fractions. Deviations from 100% represent destruction of activity, loss into other fractions, and the collected errors of these tests. The latter cause probably is the most significant. Errors in antibody assay are most serious when the activity is low, such as is found in plasma, and Fractions III-0, III-2 and III-3.

Table VII summarizes a much larger number of tests made on Fractions II-1,2, II-3 and III-1. The antibody content of the fraction, compared

TABLE VII

AVERAGE ANTIBODY CONTENT OF FRACTIONS II-1,2, II-3 AND III-1

Comparison with equal weights of plasma protein*

	II-1,2	II-3	III-1
Isoagglutinins	(0.2)	(0.4)	16
Typhoid O agglutinin	0.4	1.2	16
Typhoid H agglutinin	8	8	4
Influenza A, Hirst test	4	4	3
Influenza A, mouse protection	9	12	4
Influenza A, complement fixation	7	(7)	..
Mumps, complement fixation	8	(8)	..
Diphtheria antitoxin	10	7	4
Streptococcal antitoxin	9	9	..

* Antibody concentration of solutions of Fractions II-1,2 or II-3 containing 165 mg. protein/ml., compared with plasma, will be 2.5 times these values. Values for Fraction II-1,2 compared with plasma from Enders[4] and additional data obtained more recently (about 30 preparations). Values for Fraction II-3 were obtained largely by comparison of Fraction II-3 with II-1,2 (about 13 preparations). Values for Fraction III-1 compared with plasma or Fraction II-1,2 and II-3 obtained in about 4 preparations.

(60) We wish to thank Professor John F. Enders and his group in the Department of Bacteriology and Immunology at the Harvard Medical School—Miss Julia C. Sullivan, Miss Bettie Griffith, and Miss Jeanne E. Beauchamp—for making most of these studies. Studies of anti-A, anti-B, anti-Rh$_0$, anti-Rh', and anti-Rh" isoagglutinins were made by Dr. L. K. Diamond, Children's Hospital, Boston, Dr. W. C. Boyd, Boston University School of Medicine, Boston, and some of us (M. Melin, J. W. Cameron, and P. A. Ricketts). The distribution of the following antibodies has been studied: (a) On most preparations: agglutinins against typhoid O antigen; agglutinins against typhoid H antigen; antibody causing inhibition of the hemagglutinin of influenza A virus, strain PR8; mouse protection (neutralizing) antibody against influenza A virus, strain PR8; diphtheria antitoxin (interdermal neutralization test on rabbits). (b) On many preparations: complement-fixing antibody against the virus of mumps; complement-fixing antibody against influenza A virus, strain PR8; (c) on selected preparations: agglutinins against H pertussis, phase I, strain 484, Philadelphia; streptococcal antitoxin (interdermal neutralization test on rabbits); antibody against streptococcal hemolysin; isoagglutinins against cells of human blood groups A and B; isoagglutinins against cells of human blood groups Rh$_0$, Rh' and Rh".

with the content of the same weight of plasma protein has been recorded.

TABLE VIII

ASSAY FOR PROTHROMBIN AND PLASMINOGEN ACTIVITY
Values are in units/cc. plasma

Run	Plasma	II + III	II + III Susp.	II + IIIW	III	III-2	III-3
		Prothombin					
171	82	63	75	70	46	..	..
173	100	54	91	60	..	..	..
175	...	95	89	50	41	..	..
176	58	83	80	60	37	..	..
177	72	61	38	17	..	..	..
178–179	88	58	18	190	35	..	..
180–181	56	68	32	55	37	34	..
182–183–184	68	73	24	18	27	..	..
185–186	63	78	53	31	21	..	..
S362-1,2	...	..	62	73	81	78	..
		Plasminogen					
S-362-1,2	...	..	32	27	30	(21)	84

Typical assays for prothrombin and plasminogen activity are recorded for the various fractions in Table VIII.[61] Assays for both of these substances are somewhat uncertain, probably due largely to the presence of inhibitors which make it difficult to completely convert prothrombin to thrombin and plasminogen to plasmin without decomposition. Little or no prothrombin and plasminogen is found in any fraction other than III-2 and III-3.

(61) These studies have been made by Miss S. G. Miller and one of us (D. A. Richert), under the supervision of Professor J. T. Edsall. The methods used are recorded elsewhere.[56,58]

Summary

1. The properties of various protein components of Fraction II + III of normal human plasma are reviewed.

2. Methods are outlined for the separation of Fraction II + III into a series of subfractions by low-temperature ethanol precipitation, in which careful control of the pH, temperature, and concentration of ethanol, salt and protein have been achieved. The subfractions so obtained have led to satisfactory separation and concentration of the following components: γ-globulin antibodies in Fraction II, isoagglutinins in Fraction III-1, prothrombin in Fraction III-2, plasminogen in Fraction III-3 and β_1-lipoprotein in Fraction III-0.

3. Studies of the distribution of components into these subfractions are tabulated. They have involved measurements of protein nitrogen, dry weight of protein, cholesterol, prothrombin activity, plasminogen activity and various antibody activities.

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Filter Paper Chromatography

BY HENRY B. BULL, J. WILFRID HAHN AND VICTOR H. BAPTIST

Consden, Gordon and Martin[1] reported a very ingenious method for the separation of amino acids by filter paper chromatography in which phenol or some other appropriate organic solvent sweeps the applied amino acids along the filter paper, capillarity causing the organic solvent to move. The positions of the amino acids are located by spraying the filter paper with a solution of ninhydrin and heating the filter paper strips. The amino acids develop colors, the intensity and tint of which depend upon a number of factors. A review of filter paper chromatography has recently appeared.[2]

The present paper reports an attempt to make filter paper chromatography of amino acids quantitative. The percentage light intermission along the chromatogram is measured and this transmission has been plotted on semi-log graph paper against the distance along the filter paper strips, and the areas of the segments above the plotted curve measured with a planimeter. It has been found that the areas so determined are over a limited concentration range a simple function of the concentration of the amino acids. The various factors which influence the reliability of this method are reported.

(1) Consden, Gordon and Martin, *Biochem. J.*, **38**, 224 (1944).
(2) Consden, *Nature*, **162**, 259 (1948).

Experimental

Carl Schleicher and Schuell quantitative filter paper number 507 was cut against the machine direction into strips 7.20 mm. wide and 60 cm. long. One end of the filter paper strip was placed in the bottom of an oblong butter dish and a thick glass strip placed on top of the paper to hold it in position. It was found convenient to use 5 such strips hanging at about 2 cm. intervals over each side of the butter dish. The lower ends of the filter paper strips were attached to small metal clamps affixed to a metal rod. 0.0135 cc. of the amino acid solution adjusted to a pH 5 to 7 was placed on a marked spot on the filter paper strip with a Blodgett pipet about 4 cm. from the edge of the butter dish. The filter strips were in a horizontal position during this operation. The amino acid solutions were air dried on the filter paper and the butter dish, with the filter paper strips hanging vertically, was placed on a stage in a tall glass jar. The jar had a layer of 80% aqueous solution of Merck reagent grade of phenol on the bottom. Eighty per cent. aqueous solution of phenol was poured into the butter dish to cover the ends of the filter paper strips. A second glass jar was inverted over this jar and the joint between the jars sealed with vaseline.

Literature Reference 2

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The Plasma Proteins: Their Properties And Functions[1,2]

By EDWIN J. COHN

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ASMA is one of the most important of the body fluids. Its physiological functions are of profound significance to those con- with water and electrolytes, with hormonal th protein systems; its therapeutic uses to cerned with blood coagulation, with and with the treatment of shock.

lasma contains fats and carbohydrates and w that these occur in various metabolic nd in a very labile equilibrium. The plasma s enzymes that play their complex roles in etabolic processes, and intermediary prod-

ucts of metabolism, certain of which appear to have hormonal functions. The plasma contains waste products of the tissues, as well as components for the repair of the tissues, both in amounts which vary widely about the mean which constitutes the physiological state. The plasma may contain virus proteins, other antigens of foreign origin and the antibodies to them, derived from native globulins, as well as the large number of complex proteins native to the blood.

The most mobile constituent of the blood stream is water. Water represents over ninety per cent of normal plasma. Changes in water balance in the body are reflected by changes in the water content of the plasma. The red blood corpuscles, as well as the other body tissues, are in equilibrium with the plasma, and changes in the volume of these red cells—or of the concentration of the hemoglobin that they contain—are commonly employed as an indication of changes in the body fluids and tissues. Thus, hemoconcentration, a frequent concomitant of shock, clearly recognized by Starling, generally indicates passage of water from plasma to the tissues. One form of therapy has consisted in the injection of plasma proteins with the intent of withdrawing water from the tissues, thus effecting a hemodilution in order that the blood may again approach its normal physiological properties and its normal circulation be restored.

Hemodilution follows the injection of concen-

[1] ...erenga Prize Lecture III (Award XXXVII), ...ollege of Physicians of Philadelphia. Read at ...ege, October 14, 1942, before a joint meeting of ...llege and The Philadelphia County Medical ... Lectures delivered before joint meetings of ...rganizations may appear either in the *Transac-* ... *Studies* or in the Society's publication, *Phila-* ... *Medicine*, or in both. This lecture will appear ... the *Transactions & Studies*.

[2] ...e pressure of the times has prevented the prepa- ... of a comprehensive bibliography. References ...en to the source of the measurements reported ...les and figures. For a partial list of those who ...ade valuable contributions to this field see *Chem.* ...8-305 (1911), and a bibliography prepared for ...per by Cohn, E. J., Oncley, J. L., Strong, L. E., ...rong, S. H., Jr., Ferry, R. M. and Hughes, W. L. ..."Blood Substitutes and Blood Transfusion," C. ...omas, Springfield, Illinois (1942), pages 180–183.

trated salt or glucose solutions, but the osmotic effect of these small molecules is of short duration, since they readily leave the blood stream, through the capillary walls, which are permeable to molecules of this size. The molecular weight of glucose is 180.0, of the chloride ion 35.5, of the sodium ion 23.0, and of water 18.0. The volumes and effective diameters of such molecules are small in comparison with those of the plasma proteins. Thus the molecular weight of serum albumin is close to 70,000; that of most serum globulins is in the neighborhood of 150,000; and certain of the serum globulins of significance in immunity have molecular weights of 900,000 or more. Molecules of the size of the plasma proteins do not normally pass through the walls of blood vessels in large amount, or through the kidney, although they may, of course, do so under conditions of altered permeability.

The chemist does well in planning his work to defer to the philosopher. The philosophy of the chemist—in the twentieth century—has often been mechanistic and so has that of many physiologists. But the thinking of some physiologists has not been uninfluenced by teleology; the theory which assigns a definite end or ends in explanation of the structure and behavior of things.

If the chemist is to develop a therapeutic agent whose function is to draw water into the blood, and restore its effective circulation, what physical properties should he as a teleologist specify for the blood substitute?

THE CONCEPT OF ISOTONIC SOLUTION

Nineteenth-century botanists considered the forces necessary to draw water into tissues. Following the pioneering studies of Pfeffer and of de Vries on the passage of water through membranes, van't Hoff developed the concept of osmotic pressure. The theory of osmotic pressure that was first suggested has been superseded. The phenomenon, first studied extensively in the last century in terms of the rupture of plant cells and of red blood cells, remains, however, the most important that we know in connection with water balance.

Plant cells, and animal cells, are surrounded by membranes. These membranes are impermeable to the substances of larger molecular weight which make up the greater part of the constituents of such cells, but are permeable to water and to many smaller molecules. It is the substances that cannot pass through the cell walls into the body fluids which cause the water in our body fluids to penetrate the cell walls and dilute them. The force or pressure with which the non-diffusible contents of cells attract water was called osmotic pressure by the early workers in the field. Let us reconsider the classical studies of Pfeffer upon osmotic pressure.

Pfeffer made an artificial cell through which water could pass, but through which molecules of sugar could not pass. He made his cell by depositing copper ferrocyanide in the pores of a clay vessel. The membrane was the precipitate of copper ferrocyanide, and the clay walls functioned merely to give strength in order that the membrane might withstand the pressures that were developed. Pfeffer connected such an artificial osmotic cell containing a sugar solution to a manometer and immersed the cell in a beaker of water. The sugar solution could not pass the walls of the cell and was accordingly prevented from diffusing equally through the contents of the beaker, as it would have done if no membrane had been present. As a result the water passed through the walls of the membrane to dilute the sugar solution, and the pressure developed in this way could be read upon the manometer. Pfeffer discovered that the pressure developed by a sugar solution was almost exactly proportional to the concentration of sugar within his membrane. The results of some of his experiments published in 1877 are reproduced in Table I.

TABLE I

Osmotic Pressure of Sugar Solutions (*Pfeffer*)*

Concentration	Pressure	P/C
1%	535 mm.	535
2%	1016 mm.	508
4%	2082 mm.	521
6%	3075 mm.	513

* Pfeffer, W., "Osmotische Untersuchungen-Studien zur Zellmechanik," W. Engelmann, Leipzig (1877), p. 81.

And now we must consider those developments arising from Pfeffer's experiments which concern us still more closely: the osmotic pressure of cells. Stimulated by Pfeffer's experiments, de Vries began to study the plasmolysis of plant cells and Hedin and Hamburger the laking of red blood cells. They immersed red blood cells in sugar solutions and in solutions containing different concentrations

discovered that if solutions were en- ne strength was greater than a certain red blood cells would lose water, and, quence, would shrink. On the other he red blood cells were immersed in ter or in dilute salt solutions they would ater from the environment, burst cell the hemoglobin would flow out. The aked. Salt solutions sufficiently dilute ells to imbibe water from them were potonic, and salt solutions so strong as t water from cells hypertonic. Salt r sugar solutions which caused no change lume of the red blood cell were termed *iso*. Such salt solutions were considered to be rious with the contents of the cell.

s of experiments with varying salt con- n carried out by Hedin at this time is f able II. If he used a salt solution that ilute, the red blood cells would take up ell and increase in size, whereas in con- salt solution they would shrink.

ther tissues were subject to the same laws ic pressure relating concentration of salt as to water balance was soon recognized. 1913, quotes some experiments of Webster gastrocnemius of the frog immersed in ypo- and hypertonic solutions. For the as for the red blood cell, there is an equi- condition in which there is no exchange of tween the tissue and the solution which is it.

ese early experiments on the change in of erythrocyte and muscle cells with change r sugar content of their environment, the the cell membranes remained impermeable large structural elements within the cells, hemoglobin of the red blood cell, to the proteins of the gastrocnemius. The sugar n in which the cells were immersed freely through the walls of these cells, but were d at the concentrations at which they were by the laboratory vessels in which the ex- nts were carried out. They could not pass h the wall of the beaker, they could not into tissue spaces, readily pass the kidney c the body through the oozing surface of a

salt or sugar is dissolved in water within a rane immersed in a beaker of water and the rane is permeable to the dissolved substance will be two opposing processes simultaneously proceeding. 1) Water will flow through the membrane to dilute the dissolved substance. 2) The dissolved substance will diffuse through the membrane into the outer liquid. The rate at which these two processes will take place will depend upon the diffusion rate, respectively, of the solvent and of the dissolved substance. The

TABLE II

*The Effect of Hyper- and Hypotonic Solutions upon the Volume of the Red Blood Cells (Hedin)**

Tonicity of Solution	Concentration of NaCl	Per cent of NaCl	Volume of Red Blood Cell
Hypotonic	0.08	0.45	50.2
Hypotonic	0.12	0.67	44.2
Isotonic	0.16	0.90	40.4
Hypertonic	0.20	1.12	38.0
Hypertonic	0.24	1.35	36.8

* Hedin, S. G., *Skand. Arch.*, 5, 238 (1895), quoted by Loeb, J., *Science*, 37, 427 (1913), from more extensive data.

TABLE III

*The Effect of Hyper- and Hypotonic Solutions upon the Gastrocnemius of the Frog (Webster)**

Tonicity of Solution	Concentration of NaCl	Per cent of NaCl	% Change in Wt. of Muscle	Concentration of Sugar	% Change in Wt. of Muscle
Hypotonic	0.033	0.016	+25	0.05	+26
Hypotonic	0.065	0.032	+13	0.12	+11
Isotonic	0.125	0.065	0	0.23	−1
Hypertonic	0.25	0.13	−7	0.45	−12
Hypertonic	0.5	0.27	−13	0.87	−27
Hypertonic	M	0.54	−17	1.67	−40

* Webster, H. W., Univ. of Chicago, Decennial Publications, 10, 105 (1903), quoted by Loeb, J., *Science*, 37, 427 (1913).

pressure, high at first, will diminish as the solute passes into the outer liquid, and at the time that concentration of the solute is equal on both sides of the membrane the solutions will be isosmotic. No force will remain drawing water within the membrane.

The blood vessels are permeable to water, glucose and sodium chloride. Although there will thus

be an immediate effect of hemodilution following the injection of a concentrated salt or glucose solution, this effect will diminish with time. The constant which defines the rate of diffusion in water at 20° is 139×10^{-7} for sodium chloride and 64×10^{-7} for glucose.

The smaller the diffusion constant of the injected substance, the more prolonged will be the hemodilution, that is, for the case of diffusible substances, of substances to which restraining walls are permeable. The diffusion constants of the plasma proteins are of a different order of magnitude from salt and sugar. The diffusion constant of serum albumin is approximately 6×10^{-7} and of serum globulin 4×10^{-7} at 20°. Under conditions of free diffusion their rate of movement is thus ten or fifteen times as slow as glucose or twenty to thirty times as slow as sodium chloride.

OSMOTIC PRESSURE OF PLASMA AND OF THE PLASMA PROTEINS

The plasma proteins do not pass freely through the blood vessels and kidney under normal conditions. Their function is to be compared therefore less with that of freely permeable smaller molecules than of the substances of tissues which do not diffuse from the intact cell; with the hemoglobin of the red cell (see Table II) and with the proteins of the muscle (see Table III). Each of these substances of large molecular weight also exerts osmotic pressure, however, and since this osmotic pressure will not diminish with time, due to diffusion through the cell walls to which they are impermeable, the force which they exert is sometimes termed "colloid osmotic pressure," or, more recently, "oncotic pressure." But this is the original definition of osmotic pressure, and one to which we may well revert. An early attempt to relate the osmotic pressure of plasma to the average molecular weight of the plasma proteins was made by Starling before the turn of the century.

Osmotic pressure is as a first approximation dependent upon the number of molecules or ions in a given volume of solution. The osmotic pressure of equal weights of sodium and chloride ions, of glucose and of serum albumin through membranes impermeable to all of them would be, as a first approximation, inversely proportional to the molecular weights of the substances. This is sometimes called the "total osmotic pressure", and may be calculated from freezing point or vapor pressure measurements. In cases in which membranes are freely permeable to the smaller molecules, the osmotic pressure of salt and sugar at equilibrium may be taken as close to zero and their effects on therapy are therefore determined largely by their diffusion constants.

The smaller the diffusion constant the larger the molecular weight and therefore the smaller the osmotic pressure. For the case of molecules to which cell walls are not permeable therefore the smaller the molecular weight and the larger the diffusion constant, the greater the osmotic effect. There is, however, a lower limit to the size of a protein that can exert steady osmotic pressure; namely, the size of molecules that will not too readily be lost from the blood stream. We know that Bence-Jones protein passes into the urine, and its molecular weight is under 40,000. Egg albumin, recently studied by Bott and Richards, passes through the glomerular filter, though not freely, and its molecular weight is now estimated to be just over 40,000. The molecular weight of hemoglobin is generally given as 68,000, or close to that of serum albumin. It breaks down into molecules of half this size under a variety of conditions which appear not to affect the serum albumin molecule, however; though it has not been proven conclusively that this difference is of physiological significance.

The colloid osmotic pressure of the plasma is exerted almost completely by the plasma proteins, and their molecular weights are now relatively well known, both as a result of osmotic pressure and of diffusion and ultracentrifugal measurements. Approximat ly sixty per cent of the protein of normal human plasma is albumin of molecular weight near 70,000. Of the globulins, the molecular weights of all but a few per cent fall between 140,000 and 200,000.

The largest component thus far studied is the globulin, which is elevated in sera immune to pneumonia, which aggregates so as to yield molecules of even larger molecular weight, especially in the case of horse antipneumococcus sera (Table IV). There is also an α-globulin fraction which appears to dissociate into molecules approximately the size of the albumins (Table V).* The importance of

* In the absence of more precise knowledge of the normal concentration—and concentration in pathological conditions—of the dissociated and aggregated serum globulins we may tentatively assume, for convenience, that the changes in osmotic pressure due to these changes in molecular weight balance each other.

plasma should thus exert at least three-quarters of its colloid osmotic pressure. As we shall see, it exerts slightly more as a result of other properties of the molecules.

The osmotic pressure of purified albumins, both of human and animal plasmas, has recently been very accurately measured by Scatchard, Batchelder and Brown. Their measurements in dilute protein solution are graphically represented in Figure I. The ratio of the osmotic pressure to the concentration of the protein (P/C) is plotted as the ordinate against the concentration of protein as abscissa, concentration being in grams per kilogram of water.

Over this concentration range, all the measurements fall on straight lines which extrapolate to the same origin. The value of the origin permits a calculation of the molecular weight of serum albumin as 68,800, in very good agreement with the value deduced from sedimentation and diffusion measurements.

These measurements permit three other deductions to be made. 1) From the point of view of osmotic pressure there is little difference in the properties of human and bovine serum albumin. 2) The osmotic pressures of these albumin solutions reflect forces not observed in Pfeffer's study of sugar solutions which cover the same concentration range (Table I). The ratio (P/C) is not constant but increases with increasing protein concentration. 3) The slope of the straight lines in Figure I is greater near the pH of the blood than near the isoelectric point of the protein. That is to say, the effect of each gram of albumin on osmotic pressure is greater the greater the albumin concentration and the higher the valence of the albumin, that is, the more of it is present as a sodium salt.

TABLE V

Dimensions of Bence-Jones Proteins, Normal and Dissociated Hemoglobin, Egg Albumin, and Denatured Serum Albumin

	Diffusion Constant	Sedimentation Constant	Molecular Weight	Approximate Dimensions	
				Length of Ellipsoid	Equatorial Diameter of Ellipsoid
	$D_{20,w} \times 10^{7}$	$S_{20,w}$	M	Ångstrom Units	
Denatured Serum Albumin in Urea (Horse)	4.2[a]		67,000[a]	330[a]	20[a]
Dissociated Serum Globulin (Human)		3.6[b]	(80,000)[c]	(310)[c]	(24)[c]
Normal Carboxyhemoglobin (Horse)	6.3[d]	4.4[d]	68,000[d]	155[h]	33[h]
Dissociated Hemoglobin (Horse)	7.8[e]	3.2[e]	39,000[e]	118[h]	29[h]
Egg Albumin	7.2[d]	3.6[d]	44,000[d]	124[f]	31[f]
Bence-Jones Protein, α		3.7[g]	35,000[d]	43[h]	43[h]
Bence-Jones Protein, β	7.3[d]	2.8[d]	38,000[d]	144[h]	25[h]

[a] From Neurath, H. and Saum, A. M., *J. Biol. Chem.*, 126, 347 (1939). A similar diffusion constant and larger molecular weight (78,000) were reported by Neurath, H., Cooper, G. R., and Erickson, J. O., *J. Biol. Chem.*, 142, 249 (1942). These later results would lead to somewhat greater symmetry, with maximum values of equatorial diameter of ellipsoid equal to about 27 Å, and length 360 Å.

[b] From Neurath, H., *J. Am. Chem. Soc.*, 61, 1841 (1939). The method used assumes that the ellipsoid must be elongated and neglects any hydration of the molecules, thus representing a maximum amount of asymmetry.

[c] Value of sedimentation constant is that observed for one component of α-globulin from horse serum (Cohn, E. J., McMeekin, T. L., Oncley, J. L., Newell, J. M., and Hughes, W. L., Jr., *J. Am. Chem. Soc.*, 62, 3386 (1940)). Molecular weight is assumed to be approximately half of the figure usually given for normal serum globulin. The dimensions are calculated by the method described in reference *b*.

[d] From Svedberg, T., and Pedersen, K. O., "The Ultracentrifuge," Table 48, p. 406, Oxford University Press, Oxford (1940).

[e] Values obtained by Steinhardt, J. (*J. Biol. Chem.*, 123, 543 (1938)) from experiments in concentrated urea solutions.

[f] Computed for an axial ratio, a/b, of 4 and a hydration of 0.2 gram of water per gram of protein. (Oncley, J. L., *Ann. N. Y. Acad. Sci.*, 41, 121 (1941).)

[g] Unpublished measurements of Mehl, J. W. and Oncley, J. L. These values give the same molecular weight as observed in reference *d*, but a slightly greater frictional ratio f/f_0.

[h] Computed for an axial ratio, a/b, of 4 and a hydration of 0.2 gram of water per gram of protein. (Oncley, J. L., *Ann. N. Y. Acad. Sci.*, 41, 121 (1941).)

[i] From Neurath, H., *J. Am. Chem. Soc.*, 61, 1841 (1939). The method used assumes that the ellipsoid must be elongated and neglects any hydration of the molecules, thus representing a maximum amount of asymmetry.

[j] From Cohn, E. J., McMeekin, T. L., Oncley, J. L., Newell, J. M., and Hughes, W. L., *J. Am. Chem. Soc.*, 62, 3386 (1940).

[k] Computed for an axial ratio, a/b, of 8 and a hydration of 0.3 gram of water per gram of protein. (Oncley, J. L., *Chem. Rev.*, 30, 433 (1942).)

[l] The dimensions are calculated by the method described in note g.

[m] Tentative value for bovine fibrinogen reported by Cohn, E. J., *Chem. Rev.*, 28, 395 (1941).

[n] Estimated from sedimentation constant and viscosity measurements. This value only indicates that the molecules are quite long.

[o] Results reported on dried cells.

[p] Results reported on wet cells.

[q] These are the diameters of the sodium and chloride ion, respectively, as given by Pauling, L., "The Nature of the Chemical Bond," Cornell University Press, Ithaca, New York, 1939; 2nd ed., 1940.

studying the variation in concentration of these proteins of variable molecular weight, both in the laboratory and in the clinic, can scarcely be overemphasized.

On the basis of their size alone the osmotic pressure due to the albumins should be more than twice as great[a] as that due to the other plasma proteins. The sixty per cent of albumin in human

TABLE IV

Dimensions of Plasma Proteins, Sodium Chloride, Glucose and Red Blood Cells

	Diffusion Constant	Sedimentation Constant	Molecular Weight	Approximate Dimensions	
				Length of Ellipsoid	Equatorial Diameter of Ellipsoid
	$D_{20,w} \times 10^{7}$	$S_{20,w}$	M	Ångstrom Units	
Water			18.0	3.0[q]	
				1.9[r]	
Sodium Chloride	139[q]		58.5	3.6[s]	
Glucose	64[b]		180.1	7.2[n]	
Serum Albumin:					
Horse	5.8[a]	4.1[a]	68,000[a]		
	6.1[d]	4.5[d]	70,000[d]		
Bovine	6.3[a]	4.5[a]	68,000[a]	150[d]	35[d]
Human	6.1[a]	4.6[a]	72,000[d]		
Serum Globulin:					
Total Globulin (Horse)	4.0[d]	7.1[d]	167,000[d]	280[n]	37[n]
γ Pseudoglobulin (Horse)	4.1[a]	6.3[a]	142,000[a]	330[d]	42[d]
γ Serum Globulin (Human)	3.8[d]	7.1[d]	176,000[d]	320[d]	36[d]
Antipneumococcus (Human)	3.6[d]	7.4[d]	195,000[d]	350[n]	37[n]
Anti-pneumococcus (Horse)	1.8[d]	19.3[d]	910,000[d]	950[n]	47[n]
Fibrinogen		7.6[b]		(1000)[m]	
Red Blood Cells				20,000[o]	76,000[o]
				26,000[p]	86,000[p]

[a] Calculated from molal volume, assuming spherical shape.

[b] From International Critical Tables, McGraw Hill, New York (1929).

[c] Unpublished measurements of Mehl, J. W. and Oncley, J. L. These values give the same molecular weight as observed in reference *d*, but a slightly greater frictional ratio f/f_0.

[d] From Svedberg, T., and Pedersen, K. O., "The Ultracentrifuge," Table 48, page 406, Oxford Press, (1940).

[e] Unpublished measurements of Oncley, J. L.

[f] Computed for an axial ratio, a/b, of 4 and a hydration of 0.2 gram of water per gram of protein. (Oncley, J. L., *Ann. N. Y. Acad. Sci.*, 41, 121 (1941).)

[g] From Neurath, H., *J. Am. Chem. Soc.*, 61, 1841 (1939). The method used assumes that the ellipsoid must be elongated and neglects any hydration of the molecules, thus representing a maximum amount of asymmetry.

[h] From Cohn, E. J., McMeekin, T. L., Oncley, J. L., Newell, J. M., and Hughes, W. L., *J. Am. Chem. Soc.*, 62, 3386 (1940).

[i] Computed for an axial ratio, a/b, of 8 and a hydration of 0.3 gram of water per gram of protein. (Oncley, J. L., *Chem. Rev.*, 30, 433 (1942).)

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[l] Estimated from sedimentation constant and viscosity measurements. This value only indicates that the molecules are quite long.

[m] Results reported on dried cells.

[n] Results reported on wet cells.

[o] These are the diameters of the sodium and chloride ion, respectively, as given by Pauling, L., "The Nature of the Chemical Bond," Cornell University Press, Ithaca, New York, 1939; 2nd ed., 1940.

These effects become even more pronounced in more concentrated albumin solutions in which the linear relation between (P/C) and C does not obtain. (Figure II.) Over this range also osmotic pressure measurements demonstrate the very comparable osmotic properties of bovine and human serum albumins.

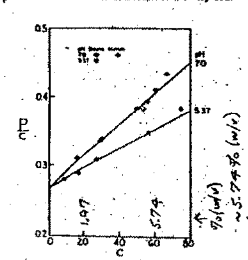

FIG. I. Serum albumin in 0.15 M NaCl. Pressure-concentration ratio vs. concentration.

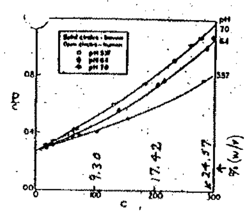

FIG. II. Serum albumin in 0.15 M NaCl. Pressure-concentration ratio vs. concentration.

The greater osmotic pressure of the sodium salt than of isoelectric albumin is illustrated in Figure III, in which the ordinate is the value of (P/C) in 6% solution and the abscissa pH.

These measurements were carried out in the presence of an isotonic sodium chloride solution. The values in Table VI are close to those which may be expected to obtain under physiological conditions (although the temperature of these experiments for technical reasons was maintained at 25° C.). The total as well as the colloid osmotic pressures have been calculated and are recorded in the table, since they indicate immediate tendencies, though not the colloid osmotic pressures upon which protracted osmotic effects depend. Whether the immediate effects of high salt concentration are beneficial or deleterious in shock is a clinical problem, which is probably more related to effects of the salt other than those on water balance.

The osmotic pressures of serum and plasma, as well as of 4 times concentrated plasma, are also reported in Table VI. The "total osmotic pressure" of a 4 times concentrated plasma is, of course, far greater than that of the other protein solutions, since in concentrating a plasma the salt is also increased to 4 times the physiological concentration. This is not true of the 25% albumin solutions which can be prepared containing any kind and concentration of salt or of other components which may be found advantageous, either for its stability or for its therapeutic effects.

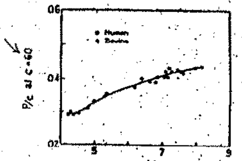

FIG. III. Serum albumin in 0.15 M NaCl. Pressure-concentration ratio vs. pH.

The simplest generalization that can be made from these osmotic pressure measurements, which confirms certain earlier studies, notably those of Peters, is that the albumins, although present in plasma to only approximately 60%, are responsible for approximately 80% of the osmotic pressure of human plasma.

LOSS OF PROTEINS FROM THE PLASMA

Is albumin more likely than globulin to leave the blood stream? In the case of a small change in permeability in the surrounding membranes, it surely is. Recent studies, both dielectric and electrophoretic, of the distribution of the plasma proteins not alone in urine but in body fluids, carried out in Sweden, at the Rockefeller Institute, and in our laboratory, suggest, however, that the old notion of albuminuria must be revised. Generally a number of different plasma proteins, characteristic of the pathological condition, pass from the blood stream. The electrophoretic distribution of protein components appearing in the urine under certain pathological conditions, is more nearly that of normal plasma than is the distribution in the pathological plasma itself. This implies, to sure, a greater loss of albumin from the plas than of globulin, especially in the case of albumin-poor plasma. In the case of serous ef sions also, "albumin is present in the same higher proportion, in the effusion, as compa

TABLE VI

Osmotic Pressure of Serum, Plasma, and Plasma Proteins

	Total Osmotic Pressure in Solutions of		Colloid Osmotic Pressure-Conc'n. Ratio in 0.15 M NaCl, pH 7.4, (P/C) for C = 0	Colloid Osmotic Pressure of Protein Solutions in 0.15 M NaCl, pH 7.4	
	6%	25%		6%	25%
	mm. Hg	mm. Hg	mm. Hg/gm. 100 cc.	mm. Hg	mm. Hg
NaCl	37,000[a]	206,000[a]			
Glucose	6,400[b]	33,100[b]			
Serum Albumin:					
Horse			2.6[c]		
Bovine	5,700[d]	8,200[d]	2.7[d]	27[d]	350[d]
Human	5,700[d]	9,100[d]	2.7[d]	27[d]	355[d]
Total Globulin:					
Bovine			1.1[e]	6.5[e]	
Fibrinogen			0.9[e]		
Serum			2.2[d]	21[d]	
Plasma	6,200[f]		2.1[d]	20[d]	
Plasma, 4X Concentrated		33,000[g,h]			280[g,e]
Blood				20[f]	

[a] On basis of total osmotic pressure. Calculated from molecular weights.
[b] International Critical Tables, IV, 430.
[c] Adair, G. S., and Robinson, M. E., *Biochem. J.*, 24, 1864 (1930). Phosphate buffer solutions were used as t[illegible] solvent.
[d] Scatchard, G., Batchelder, A. C., and Brown, A. Buffalo meeting, Am. Chem. Soc., September, 1942. Al[illegible] unpublished results. The total osmotic pressures of bovine and human albumin solutions were calculated fro[illegible] the freezing points of solutions prepared at the Department of Physical Chemistry, Harvard Medical Scho[illegible] The colloid osmotic pressure of 25% bovine albumin solution is estimated from measurements at pH 7.0, [illegible] electrolyte, 0.15 M NaCl.
[e] Scatchard, G., Batchelder, A. C., and Brown, A. Unpublished results. A preliminary estimate fr[illegible] measurements on 2.2% solutions in 20% glycerol, in the presence of sodium phosphate buffers, pH 7.0, Γ/2 = 0[illegible]
[f] For 6% plasma proteins. The osmotic pressure of blood and of plasma are taken as equal at equal plas[illegible] protein concentrations.
[g] Calculated from unpublished measurements by Scatchard, G., Batchelder, A. C., and Brown, A., of [illegible] freezing points of solutions of commercial dried plasma.
[h] The salt is also concentrated fourfold.

INFLUENCE OF THE SHAPE OF PROTEIN ON THE VISCOSITY OF SOLUTIONS

The viscosity of solutions is influenced far more by the shape of molecules than by their size. Indeed, the simple theory, which cannot be completely applicable to protein solutions such as plasma—and far less so to blood—demands that the viscosity of solutions of spherical, incompressible, uncharged substances be the same, regardless of their size, provided they occupy the same volume fraction. Solutions of fibrinogen and the globulins are more viscous than those of albumins, not because the molecules are larger but because they are more asymmetrical. The ratio of the axes in the case of the serum proteins that have thus far been most completely studied by various methods, namely the serum albumin and γ pseudoglobulin of the horse, is given in the first column of Table VII. The evidence thus far available suggests that the shape as well as the size of the comparable proteins of human and animal plasmas is very similar. The relative viscosities of solutions of the albumins of different species have been studied and are compared with the viscosities on the one hand of salt and glucose solutions, on the other with that of human serum, plasma and blood.

Blood, plasma and serum are all more viscous than albumin, but less viscous than globulin or fibrinogen solutions of the same concentration. Thus the viscosity of blood with hematocrit 40 is of the same order of viscosity as 6% plasma, that of serum and serum albumin is far smaller than even a 2.2% solution of fibrinogen. These more asymmetric proteins, despite their smaller concentration in plasma, thus exert a greater effect on viscous than on osmotic properties. This becomes especially noteworthy in the comparison of hypertonic albumin and plasma. Fourfold concentrated plasma is approximately ten times as viscous as normal plasma and almost three times as viscous as a 25 per cent human serum albumin solution.

TABLE VII

Relation of Molecular Shape to Viscosity of Protein Solutions

	Ratio of Axes	Viscosity Coefficient	Relative Viscosity of Solutions	
			6%	25%
	a/b		η/η°	η/η°
Water				
Sodium Chloride			1.01	
Glucose		4	1.24	2.7
Serum Albumin:				
Horse	4	6.5	1.39	
Bovine	4	5.4	1.32	5.4
Human	4	5.8	1.35	6.1
Serum Globulin:				
γ Pseudoglobulin (horse)	8	10	1.9	
Fibrinogen	(30)	(70)	(4.3)*	
Serum			1.4	
Plasma			1.5	
Plasma, 4× Concentrated				16.0–26.0
Blood:				
Hematocrit 60			4.5	
Hematocrit 50			6.0	
Hematocrit 60			9.8	

*Fibrinogen is not very soluble in water or in physiological saline. This value is for a 2.2% solution in 0.6 M NaCl.

with plasma, and β-globulin and fibrinogen in lower proportion," (Luetscher). This is illustrated graphically in the accompanying Figure IV.

It would be important to know whether the deductions drawn from the study of serous effusions applied equally to the oozing from burns. I have not seen the results of any comprehensive study of this kind, but one could readily be made, and I trust will be. The recent analytical evidence suggests, however, that although there is a somewhat greater loss of albumin than of globulin or fibrinogen from the blood stream, the differential loss is far smaller than might have been expected.

The physical chemist, knowing little of the conditions for the passage of molecules through the walls of blood vessels or the kidney under pathological conditions, though he assumes altered permeability, considers also in this connection the dimensions of the molecules.

There are a number of methods, all related to the frictional resistance to the movement of large molecules in liquids, on the basis of which estimates of the shape and size of proteins have been made. These estimates differ somewhat, depending upon the method of investigation and of calculation, and depending upon whether allowance is made for water carried with the protein molecule. Nonetheless ... results from more than one laboratory are sat... orily concordant. They indicate that none of the blood proteins can be considered spherical, although albumins are more nearly so than globulins. Approximate dimensions estimated as though plasma proteins were ellipsoids of revolution suggest that they differ in length, but that the diameter of all human serum proteins thus far investigated is in the neighborhood of 40 Å.

The difference between 40 Å and the very much smaller dimensions of water, salt and sugar is striking. So also is the similarity between the diameters of albumins and globulins. Membranes that can separate these proteins from smaller molecules can readily be visualized. Differences in the rate of passage of these normal proteins through a membrane of pore size somewhat greater than that of their diameters would appear to depend upon statistical considerations related to the lengths of the molecules (see Table IV). Estimates of the dimensions of proteins, some of which are known to appear in the urine, are listed in Table V. In at least certain cases diameters of 30 Å or less have been calculated. The results are suggestive, but more evidence is needed before final conclusions can be drawn. The early study on a Bence-Jones protein appears as an exception. On the other hand, the difference in shape of the serum albumin preparations (horse) studied in Upsala and by Oncley and Mehl in our laboratory, and of the denatured serum albumin studied by Neurath at Duke University, demands further investigation. All have the same molecular weight.

If we now turn to hemorrhagic conditions in which not only proteins but also red cells escape from the blood vessels we must consider a totally different order of dimensions. Instead of molecular dimensions of the order of hundreds of Ångstroms the dimensions of a normal red cell may be taken as approximately equal to a disk with a mean diameter of from 76,000 to 86,000 Å (7.6 to 8.6 μ)

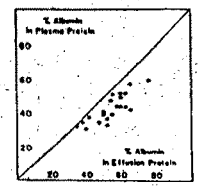

FIG. IV. The relation between the proportions of albumin in plasma and in serous effusions. From Luetscher, J. A., Jr., *J. Clin. Invest.*, 20, 104 (1941).

and with a mean thickness of from 20,000 to 26,000 Å. In the case of an opening large enough for the passage of a red cell, the difference in the sizes of albumin and globulin molecules would not be expected to have a greater influence than is indicated by the diffusion constants of the molecules; rather, one would expect it to be smaller since one is here concerned less with the diffusion of molecules than with the flow of the plasma, or rather, of the blood.

Indeed, the most asymmetric molecule of the blood stream is fibrinogen. The molecular weight of fibrinogen has not yet been accurately determined. The molecule is rod-shaped, however, having a length estimated by one method to be as great as a thousand Ångstroms. Once more the teleologist cannot resist considering the relation between the properties of this molecule and its function in the clotting of blood.

convenience and elegance of the procedure by which a plasma can be analyzed in terms of these fractions. In the accompanying figure we may compare the electrophoretic diagram of whole human plasma with that of γ and β globulin fractions purified from it. The particular γ-globulin illustrated is rich in antibodies and the β-globulin in thrombin.

TABLE VIII

Electrophoretic Analysis of Plasma Proteins, and Electromotive Force Measurements of their Valence in Blood

	Per cent of Total Protein		Electrophoretic Mobility in Phosphate Buffer pH 7.7 $\Gamma/2 = 0.2$ 8°C.	Negative Net Charge per Molecule pH 7.0	
	In Serum	In Plasma		$\Gamma/2 = 0$	$\Gamma/2 = 0.15$
Serum Albumin:					
Horse, Carbohydrate containing	42		4.5		
Horse, Carbohydrate free			5.3	17	
Bovine, Carbohydrate free	48	40	5.2	12	15
Human, Carbohydrate free	56	62	5.3	15	18
α-Globulin:					
Horse	13		3.9		
Bovine	20	16			
Human	7	7	3.8		
β-Globulin:					
Horse	21		3.9		
Bovine	10	8			
Human	14	13	2.7		
γ-Globulin:					
Horse	24		1.9	7	
Bovine	22	18	1.6		
Human	13	12	0.9		
Fibrinogen:					
Bovine		18	2.1		
Human		6	1.9		

Chemical fractionation combined with electrophoretic mobility measurements reveals more than three globulin components of plasma. Some are euglobulins, some are pseudoglobulins. Many bear prosthetic groups of diverse nature, whose structure and function is still unknown. Many may well be as important because of their specific physiological functions and perhaps because of undiscovered therapeutic uses, as are the globulins concerned with immunity.

Thus far most antibodies have been found to be γ-globulins, although some may have the mobility of β-globulins. Probably in the former group are included the antibodies to mumps and influenza, and indeed to most virus proteins. There is some evidence that the antibody to diphtheria, however, is β-globulin.

Let us turn from the consideration of antibodies to that of a few of the vast number of physiologically important protein plasma constituents that have thus far been concentrated and investigated by physical-chemical means. Thus, among proteins of importance in blood coagulation, thrombin and prothrombin are isoelectric near pH 5.2 and appear to be β-globulins. So is at least one component of the very characteristic blue-green, jelly-like euglobulin (the P_1 of Green), which also has an isoelectric point near pH 5. The so-called "mid-piece" of complement, prepared by Pillemer in our laboratory, also appeared to move in the electric field with the mobility of β-globulin and to be isoelectric between pH 5.2 and 5.4.

On the other hand, the so-called "end-piece" and "4th component" of complement, at least in the guinea pig, appeared to be α-globulin, although separating as isoelectric euglobulin, rich in carbohydrate, near pH 6.3. It is possible that the so-called mucoglobulin of Hewitt, and other carbohydrate and lipoid-rich globulins, are α-globulins. It is the α-globulins which are elevated in febrile conditions, and in lipoid nephroses, in which fibrinogen increases in the plasma as do the α- and β-globulins, whereas the albumins, and to a lesser degree the γ-globulins, are diminished. Conversely, albumin and to some extent γ-globulins are found in lymph, and perhaps in other body fluids, in higher relative concentration than in plasma. It thus would seem that certain of the γ-globulins and albumins leave the bloodstream more readily than do the α or β-globulins.

The protein composition of plasma, and the electrophoretic mobility of each of these crude fractions in a 0.2 molal phosphate buffer at pH 7.7 is given in the accompanying table (Table VIII). So also is the net charge as estimated by electromotive force measurements of those purified fractions that have thus far been studied. With the

Indeed, the 25 per cent serum albumin solution is approximately iso-viscous with blood.

Blood substitutes are prepared in concentrated form in the interest of stability, of compactness, or both. When added to the blood stream their concentration is, of course, at once diminished, first by dilution on mixing with the normal or subnormal circulating plasma, then by the hemodilution brought about by the greater osmotic pressure of a concentrated than of an isosmotic protein solution.

In the case of a failing circulation, there may well be an advantage in having the viscosity of the blood substitute as low as possible and the colloid osmotic pressure as high as will not cause hemodilution at too great an expense to critical body fluids. The final judgment as to the concentrations to bring about this optimal response can only be determined by clinical trials under varying conditions of dehydration. It cannot be deduced from the physiology of water and electrolyte balance or from the physical properties of the proteins.

Influence of the Net Charge of Proteins on the Analysis and on the Properties of Solutions

The globulins would not necessarily exert as great osmotic pressures as the albumins, even were their molecular weights the same. Their isoelectric points are more nearly neutral, and the amount of base they combine at the pH of the blood is smaller per gram, although not necessarily per mole. Thus certain of the globulins, especially those of importance in immunity, have almost neutral isoelectric points. This property, and the high molecular weight aggregates sometimes observed when this fraction is elevated in immunized animals of high antibody content, tempts the teleologist—though guardedly—to wonder whether proteins of this structure cannot give rise to the greatest interactions with other molecules, as in antibody-antigen reactions, with minimal disturbances to the water balance of the body.

The net charge of the globulins at blood pH is a property which is reflected in electrophoretic mobility measurements; though properties of molecules which influence frictional resistance also influence mobility. Tiselius termed the globulins of highest mobility α, those of lowest mobility γ globulins. The great advantage of the electrophoretic method, despite our unsatisfactory theoretical understanding of the phenomenon, is the

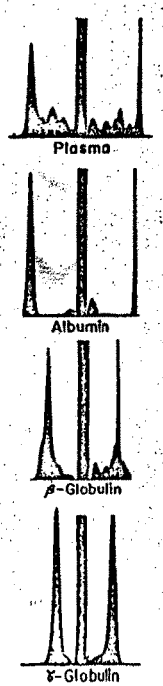

FIG. V. Electrophoretic patterns of human plasma and purified fractions.

amino acid residues, it is not impossible that their functions as immune bodies depend in part upon this greater reactivity of their molecules.

In contrast to the γ-globulin, albumins have the lowest dipole moments thus far investigated. Oncley has estimated the moment of γ-globulin to be approximately 1200 Debye units; of serum albumin approximately 400 Debyé units. We must thus conclude that the electrically charged groups of the albumin are arranged in such a way as to approximate electrical symmetry. Such a molecule should react far less than a globulin with other proteins, with neutral salts and with water. It may well be that the very high solubility of the albumins depends upon these same properties.

COMPARISON OF THE PROPERTIES OF HUMAN AND ANIMAL PLASMA PROTEINS

In the preceding discussion we have compared the properties of albumins and globulins, of human and animal origin. The degree to which the properties of human and horse γ serum globulins approximate each other is indeed striking. This is true also if we compare human, horse, and bovine serum albumin. It is true whether our criterion is electrophoretic or ultracentrifugal analysis, is diffusibility or viscosity, valence or osmotic pressure.

And yet no protein of animal origin has ever been found to be identical with the comparable human protein. The differences sometimes detectable in dielectric measurements are often reflected by changes in solubility and crystallizability. Microphotographs of crystallized human and bovine serum albumin are reproduced in the accompanying Figure VI.

Analytical evidence also reveals differences between the distribution of the residues whose spatial configurations presumably result in the species specificity of molecules. The physical-chemical behaviour of analogous proteins is thus often remarkably similar, although the fine structure of the molecules may be different.

In how far, therefore, will it ever be safe to substitute for therapy in man a protein of animal origin? This question can only be answered by clinical trial. Clearly from a physico-chemical point of view it is possible to substitute for a human protein an animal protein which will perform the same physical and physiological functions in nearly the same way. If an animal protein is to be introduced into the human body it should have the property of acting up as few as possible of the reactions to foreign proteins. Globulins, with their higher reactivity, are far less safe from this point of view than albumins. The reactions to albumin containing as little as 0.01% to 0.001% globulin are being carefully investigated, but we are not prepared to discuss the results at this time.

We have attempted to present, however, the point of view guiding the investigations which are in progress. The properties of the diverse molecules which perform the functions of the plasma must form the basis for their rational use as therapeutic agents. All of the plasma proteins, whether of human or of animal origin, can be made available, in concentrated form in any necessary state of purification or modification, and their availability should make possible a new understanding of the functions which they perform in health and disease.

exception of a part of the γ-globulin, most proteins are not isoelectric at blood pH, but exist as salts. Indeed, each mole of serum albumin may be considered combined with base, in amount which may be read directly from the titration curve or which is given in the table, both in the presence and in the absence of electrolytes. The electrochemical behavior of these and of other serum proteins is a complex function not only of the size and shape, but also of the net charge of the protein.

INFLUENCE OF THE DISTRIBUTION OF ELECTRIC CHARGES ON PROTEINS

The distribution of the charges on the surface of a molecule has but small effect on the titration curve of a protein with acid and base, or on its electrophoretic mobility. Thus the number of moles of

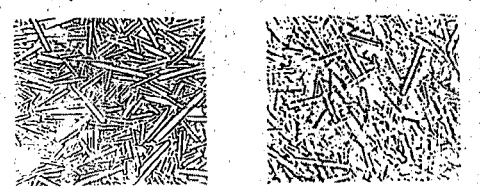

FIG. VI. Microphotographs of albumin crystals. Bovine (left), human (right).

base necessary to titrate a mole of serum albumin from its isoelectric point to pH 7.4 may be taken as 15, in the absence of electrolytes, or as 18 in physiological saline. This valence of the protein, z, is the quantity used in computing the osmotic pressure of the protein at blood pH, the quantity which determines the force which overcomes frictional resistance when the protein is in the electric field of a Tiselius apparatus.

Serum albumin has approximately 100 acid, and 100 titratable basic groups. In the isoelectric condition the molecule thus bears 100 positive and 100 negative electric charges. The negative charges are derived from dissociated carboxyl groups. The positive charges arise from guanidine, amino, and 18 imidazole groups. At pH 7.4, all the carboxyl groups are negatively charged, all the amino groups and all the guanidine groups are positively charged. At the isoelectric point all of the imidazole groups are positively charged, but at pH 7.4 all, or almost all, of them have lost their positive charge. The change in valence of serum albumin, as of most other proteins over the physiological range, is thus in large part ascribable to changes in the charged condition of the imidazole group. For the case of serum albumin about 18 positive charges are lost by imidazole groups, making a total of 82 positive and 100 negative groups per mole. The difference between these values is the net charge.

What are the effects to be expected from the other charged groups? This will depend in large part upon their distribution upon the surface of the molecule. If all the positive charges were at one end of an ellipsoidal molecule and all the negative charges at the other, we should have a very strong electric dipole. Such a dipolar ion orients in an electric field and oscillates in an alternating electric field. The electric frequency necessary for the protein to overcome frictional resistance gives information on the basis of which estimates of the shapes of molecules were deduced. More important is the magnitude of the effect which is measured by the extent to which the oriented protein increases the dielectric constant of the solution.

Theoretical considerations suggested, and experimental studies have demonstrated, that the greater this effect of proteins on the dielectric constant, the greater their dipole moments, the greater will their interactions be with salts and with other proteins. Thus, globulins whose solubility is profoundly influenced by neutral salts, have all been found to have high electric moments. Although the specificity of globulins, as antibodies, must depend upon specific spatial configurations of their

Literature Reference 3

Articles

Considerations of pool size in the manufacture of plasma derivatives

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Background: The pooling of human plasma from many donors for the purpose of manufacturing therapeutic proteins increases the risk of exposing recipients of these proteins to pathogens that may contaminate 1 or a few units included in the pool.
Study Design and Methods: This risk is estimated for a range of manufacturing scales that would derive material from a varied number of donors and for a number of hypothetical infectious agents that may exist in the donor population over a wide range of prevalence. Risk is also calculated both for recipients of single doses of a plasma protein and for those who depend on long-term treatment with plasma derivatives.
Results: Risk of exposure increases with pool size and the prevalence of the agent in question and accumulates with repeated treatments with material manufactured from different pools.
Conclusion: Reducing pool size would at best decrease this risk in proportion to the reduction in manufacturing scale. However, for individuals requiring repeated or continuous treatments, the risk of exposure to all but the rarest infectious agents would be only minimally affected, even by large reductions in manufacturing scale.

HUMAN PLASMA PROTEINS for therapeutic use have been manufactured from large pools of plasma for more than 50 years.[1,2] The original purposes of this strategy—economy and full utilization of limited resources—are still valid. The use of large pools of plasma (or the pooling of multiple manufacturing batches into larger lots) may also contribute to product consistency and improve safety in other ways. For instance, the production of immune globulin is mandated at or above a minimum scale of 1000 donors to ensure the inclusion of a broad spectrum of antibodies.[3]

Plasma proteins such as albumin, immunoglobulins, and the coagulation factors may be manufactured from source plasma[4] (approx. 500-800 mL/unit collected by plasmapheresis and restricted to further manufacturing) or from "recovered" plasma (approx. 200-250 mL/unit prepared from whole-blood donations). When these proteins are produced from source plasma, the initial pools typically include plasma from 1,000 to 10,000 individual donors. When recovered plasma is used, pools may contain plasma from up to 60,000 donors. Furthermore, during manufacture, intermediate material derived from more than one starting pool may be combined into one lot before filling the final containers. For the bulk of plasma derivatives produced in the United States, each vial contains material from more than 10,000 donors.

Recently, the wisdom of producing these products from such large pools of human plasma has been called into question. This concern centers on the possibility of transmitting viruses and other infectious agents (such as the incompletely characterized agents thought to be responsible for certain spongiform encephalopathies). Before the introduction of donor testing and virus inactivation during manufacture, coagulation factor concentrates transmitted hepatitis and human immunodeficiency viruses at higher rates than did single-donor blood components.[5,6] Even with current safety measures, however, infectious agents, such as human parvovirus B19, that are present in a plasma pool and are not inactivated during manufacturing may be transmitted to recipients of products manufactured from that pool.[7,8] The size of the pool affects this risk, because in general, more donors will contribute to larger plasma pools. The more donors who contribute to a plasma pool, the higher the probability that the pool will contain a transfusion-transmissible agent.

These considerations suggest that reducing the number of donors contributing to plasma pools from which therapeutic proteins are manufactured could reduce the risk of disease transmission by reducing the proportion of pools that include donations from infected individuals. The effect that such a change might have on the transmission of disease has been approximated by modeling the risk of exposing the recipient of plasma derivatives produced at various manufacturing scales to infectious agents that may exist in the donor population. Because

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changes in pool size would affect the risk of exposure at least as much as they would affect the risk of transmission, this approximation yields the maximum benefit that could be achieved by reducing the number of donors contributing to a plasma pool. Of course, the relationship between the risk of exposure and the actual risk of infection is complex, and no attempt has been made in this analysis to correlate the two risks. However, the mere inclusion in a plasma pool of a unit that is suspected of being infectious is sufficient to trigger the recall of products manufactured from that pool. Therefore, the risk of exposure is itself of considerable practical relevance.

Materials and Methods

Our analysis is based on the assumption that any given donor may or may not be harboring an infectious agent at the time of donation. For the sake of simplicity, it is also assumed that the infectious agent in question exists in the donor population at a steady state; that is, its prevalence adequately describes the risk associated with any single donation. If the prevalence of the agent were changing significantly with time, it would be more appropriate to use incidence to calculate this risk. However, the outcome of the analysis presented here would not be altered by substituting incidence for prevalence. Furthermore, a risk based on prevalence may be modified by donor screening, which reduces the risk of accepting a unit from an infected donor. Thus, "prevalence" as employed in the equations below would include both the prevalence of an agent for which screening is not performed and the residual risk due to false-negative results of those screening procedures that are performed.

Given these assumptions, a simple binomial model[9] describes the relationship between the size of a plasma pool and the probability of including in that pool 1 or more units containing an infectious agent that has a given prevalence in the donor population. This probability is equivalent to the risk of exposure to that infectious agent that is faced by the person who receives one treatment (single treatment) with a plasma derivative manufactured from that pool; it is calculated by the following equation:

$$\text{Risk} = 1 - (1 - \text{prevalence})^{\text{pool size}}.$$

"Pool size" in this equation is expressed as the number of donors contributing to the pool rather than the number of total units or the total volume of the pool. The risk calculated by this equation is also equivalent to the proportion of plasma pools of that size that would contain 1 or more contaminated units.

In addition to the risk of exposure associated with a single treatment, it is important to consider the cumulative risk associated with multiple treatments when the material infused is derived from several plasma pools. This cumulative risk of exposure is calculated by the following equation:

$$\text{Cumulative risk} = \text{risk of single treatment}^{\text{[number of independent infusions]}}.$$

Finally, it is possible to calculate a maximum allowable scale of manufacturing in terms of pool size to achieve a predetermined risk of exposure (i.e., targeted risk, based on a single treatment) to an infectious agent with a given prevalence in the donor population by using the following equation:

$$\text{Maximal allowable pool size} = \frac{\log_{10}(1 - \text{targeted risk})}{\log_{10}(1 - \text{prevalence})}.$$

To calculate risk of exposure or cumulative risk, the prevalence of the agent in the donor population must be known. A broad range of prevalence has been used to encompass a variety of agents that are known or suspected to be transmitted by plasma derivatives. These are assumed to be limited to small infectious agents such as viruses (and possibly the agents of spongiform encephalopathies) that are not completely inactivated or removed by chemical or physical methods commonly employed in manufacturing. Bacteria and larger pathogens are not a concern, as these would be removed by filtration. Viruses without a significant viremic phase that are transmitted only by cellular components also are not considered to pose a significant risk in this context. Of greatest concern are those agents for which no method of detection exists or for which screening for the purpose of eliminating contaminated units is not performed. To include a wide range of these agents, prevalence has been assumed to range from 1 in 5,000 (e.g., parvovirus B19[10]) to 1 in 500,000 (a rare or emerging agent).

Results and Discussion

General considerations

The risk of exposure, used in this study as the measure of risk, does not necessarily equate to the risk of infection. The infection rate depends on other variables, such as the infectiousness of the agent, the quantity of the agent in the pool, the distribution and/or inactivation of the agent during manufacturing, and the susceptibility of the recipient population. These variables, in turn, depend on the specific agent in question and the particular product being manufactured. Therefore, no attempt has been made to calculate an actual transmission rate in any of these models. In all cases, the risk of exposure is equal to or greater than the risk of infection, and so the models presented here are worst-case estimates of real risk.

In particular, two factors that may work in this context to reduce disease transmission are excluded when exposure to an agent is used to calculate risk: the dilution of the agent in the plasma pool and neutralization of the agent by antibodies. In some instances, the dilution of an infectious agent in a large pool, possibly in conjunction with other factors such as attenuation during purification, might reduce the risk of transmission. However, this effect can be quantified only for a particular agent and particular product and is implicitly excluded from the present analysis. Similarly, the risk of exposure would not include any effect of the broad spectrum of antibodies present in a large plasma pool on the transmission of the agent. There are two reasons for excluding this possibility from the model. First, although neutralizing antibodies are known to exist for a number of viruses, such as hepatitis A and parvovirus B19, those viruses may be transmitted by coagulation factor concentrates.[7,8,11-14] It is possible that the virus, which may well be complexed with neutralizing antibodies in the initial pool, dissociates from those antibodies during processing and contaminates a final product depleted of immunoglobulins, rendering that product infectious. Second, the possibility of an infectious agent yet to emerge in the plasma supply renders questions of immunity somewhat academic. The

agent might not be susceptible to neutralization by antibodies, and, if it were, little or no immunity would be expected in the donor population during its emergence.

Risk to the recipient

The risk of including in a plasma pool a unit donated by an individual carrying an infectious agent depends on the prevalence of that agent in the donor population and the size of the pool itself (Fig. 1). Pool size is defined here as the number of donors contributing to the pool. It is clear that, at large scales of manufacturing, the risk of contaminating any given pool—even with an agent whose prevalence is no greater than 1 per 10,000 donors—approaches 100 percent. Although the risk is less than 10 percent under many of the conditions included in Fig. 1, it increases dramatically as the pool size increases and as the prevalence of the infectious agent in the donor population increases.

The risk of contaminating a pool with an infectious agent is exactly equal to the risk of exposing a patient to that agent by a single treatment with a plasma derivative routinely manufactured from such pools. However, a recipient's risk may also be increased by multiple treatments with the plasma derivative in question. If all the material comes from a single lot, the risk of exposure is not changed (although the risk of infection certainly may be increased). If an individual is treated with material from different lots (independent infusions), the cumulative risk of exposure to the infectious agent is as illustrated in Fig. 2. When the risk of contaminating a single pool is low, the effect of several independent treatments is nearly arithmetic (i.e., 20 independent infusions would nearly double the risk associated with 10). As the risk associated with a single pool increases and/or the number of independent infusions increases, cumulative risk of exposure increases and then plateaus as it approaches 100 percent.

It should be noted that Fig. 2 includes only low risks of contaminating a given pool, with 10 percent being the highest category depicted in Fig. 2, whereas it is the lowest in Fig. 1. When the risk of contaminating a single pool is higher than 10 percent, cumulative risk approaches 100 percent after only a few independent infusions. Even at relatively low levels of initial risk, cumulative risk increases to near-certainty after repeated treatments. For instance, a severe hemophiliac may be exposed to 100 different lots of a coagulation factor over the course of several years.[15-17] At that level of use, the individual will face a cumulative risk of exposure of about 63 percent if only 1 percent of the plasma pools from which the factor is manufactured are contaminated with an infectious agent. For the cumulative risk associated with 100 infusions to be held below 10 percent (a modest degree of safety at best), the risk of contaminating a single plasma pool must be kept to 0.1 percent or less.

Manufacturing scale

Because patients who require long-term or lifelong treatment with plasma derivatives face an extraordinary risk of exposure to infectious agents present in the donor population, it becomes important to keep the cumulative risk as low as possible. This need, in turn, requires that very low levels of risk be associated with each individual treatment. To evaluate whether this goal can be accomplished by adjusting the size of the plasma pools used for manufacturing, it is necessary to calculate the maximum pool size that would achieve a targeted risk of exposure

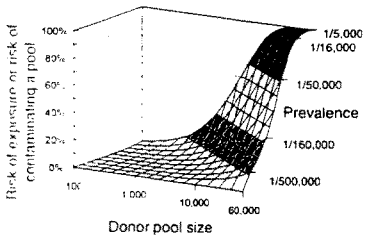

FIG. 1. Probability map of the risk of contaminating plasma pools of varied sizes, which is expressed as the number of donors (100-60,000) who have contributed to them. The hypothetical infectious agent may exist in the donor population, with a prevalence ranging from 1 per 5,000 to 1 per 500,000. The surface is shaded to indicate risk levels at 0 to 10 percent, 10 to 30 percent, 30 to 70 percent, and 70 to 100 percent. The risk of contaminating a pool is equivalent to the percentage of pools that are contaminated and to the risk of exposing a recipient of material derived from a single pool to the infectious agent in question.

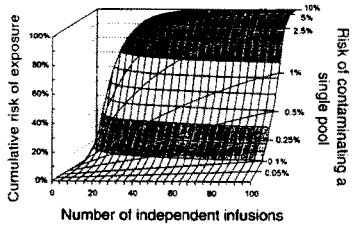

FIG. 2. The cumulative risk of exposure resulting from multiple independent infusions (treatments with material derived from different plasma pools). This risk depends on the number of such treatments and the risk associated with each treatment (risk of contaminating a pool taken from Fig. 1, but restricted here to 0.05-10%). The surface of the probability map is again shaded to indicate risk levels at 0 to 10 percent, 10 to 30 percent, 30 to 70 percent, and 70 to 100 percent.

to an infectious agent of given prevalence in the donor population. An example of this targeted risk is shown in Fig. 3. In this case, it is the risk of exposure associated with a single treatment, which, as described previously, is equivalent to the probability that a contaminated unit will be included in a manufacturing pool. The results indicate that extremely small pools—that is, pools significantly smaller than those currently used in the manufacture of most plasma derivatives—are required to achieve reasonably low risk factors. For example, manufacture at the 1,000-donor scale is allowed only if one accepts the risk that 1 percent of the pools will be contaminated by an infectious agent that exists in the donor population no more frequently than once in 100,000 donors. (As shown above, this targeted risk of 1% translates to a cumulative risk of 63% after 100 independent infusions.) To reduce this risk to 0.1 percent for the same agent, manufacture would have to be restricted to 100-donor pools. Of course, as the prevalence of the agent increases, the maximum pool size that allows the achievement of a targeted level of risk decreases still further.

An alternative approach is to determine the effect of manufacturing at different scales on the risk of exposure to a particular infectious agent faced by patients undergoing treatment with a plasma derivative once, only a few times, or chronically. Table 1 lists the results of this calculation for three hypothetical infectious agents whose prevalence in the donor population is 1 in 500,000, 1 in 50,000, and 1 in 5,000. The scale of manufacture ranges from 100 to 60,000 donors per pool. Under the most favorable circumstances—namely, a rare infectious agent and a single infusion of a plasma derivative—the risk of exposure is in almost exact proportion to the size of the pool. In contrast, the risk of exposure to the same rare agent after 100 independent infusions is decreased from 100 to 11 percent by a reduction in manufacturing scale from 60,000-donor pools to 600-donor pools. The impact of manufacturing scale, within the limits of Table 1, also decreases as the prevalence of the infectious agent increases. For the most prevalent agent included (1/5000 donors), reducing the scale from 60,000-donor pools to 600-donor pools would reduce the risk associated with a single infusion by about 90 percent (from 100 to 11%), but the cumulative risk associated with 100 infusions would not be affected to any significant degree. It must be emphasized that, for the recipient of multiple doses of a plasma derivative, the benefit of reducing pool size would be offset to some degree if the use of more numerous, but smaller lots of material exposed that recipient to a greater number of lots.

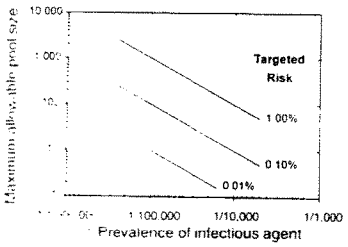

Fig. 3. The largest scale at which a product may be manufactured (maximum allowable pool size, expressed as the number of contributing donors) to achieve a given level of risk. The upper limit on the scale of manufacture depends on the prevalence of the infectious agent and the level of risk (targeted risk) that is deemed acceptable. Targeted risk is the risk of contaminating any given plasma pool or the risk of exposure to an infectious agent associated with a single treatment (see Fig. 1); it is not the cumulative risk of exposure associated with multiple treatments. Extremely small pools are required to achieve reasonably low risk factors.

Implications

The impact on safety of adjusting manufacturing scale can be no greater than the change in scale. It is frequently less than this, and, in some cases, the impact can be negligible. To effect meaningful improvements in safety by this approach would require large reductions in the scale of manufacture. For example, low rates of transmission of hepatitis, human immunodeficiency virus, and B19 have been reported in hemophiliacs treated with lyo-

Table 1. *Effect of manufacturing scale on risk of exposure*

Scale of manufacturing (number of donors)	Number of independent infusions		
	1	10	100
Prevalence of agent = 1 in 500,000			
60,000	11%	70%	100%
25,000	5%	39%	99%
10,000	2%	18%	86%
6,000	1%	11%	70%
2,500	0.5%	5%	39%
1,000	0.2%	2%	18%
600	0.1%	1%	11%
100	0.02%	0.2%	2%
Prevalence of agent = 1 in 50,000			
60,000	70%	100%	100%
25,000	39%	99%	100%
10,000	18%	86%	100%
6,000	11%	70%	100%
2,500	5%	39%	99%
1,000	2%	18%	86%
600	1%	11%	70%
100	0.2%	2%	18%
Prevalence of agent = 1 in 5,000			
60,000	100%	100%	100%
25,000	99%	100%	100%
10,000	86%	100%	100%
6,000	70%	100%	100%
2,500	39%	99%	100%
1,000	18%	86%	100%
600	11%	70%	100%
100	2%	18%	86%

philized cryoprecipitate (pool size = 6) or factor IX concentrate (pool size = 250) manufactured on extremely small scales.[18-20] Modifying current manufacturing practice to approach this scale would have significant implications for the production of these proteins. For example, even a 90-percent reduction in manufacturing scale, which would be of marginal or no benefit in most cases, would require the production of 10 times as many batches of each plasma protein in the same time to meet current clinical demand. It is unlikely, though not necessarily impossible, that current technology would accommodate sufficiently faster production of these proteins, so that parallel production would be the only viable alternative. In either case, a fundamental change in the design or a drastic expansion of the production facilities would be required. Both the cost of these proteins and the ability to produce sufficient quantities may be affected. Furthermore, the stringent standards of current Good Manufacturing Practices provide a significant element of safety for the recipients of plasma derivatives and other pharmaceuticals.[21-23] The adherence to current Good Manufacturing Practices is labor-intensive, emphasizing compliance with written procedures, testing at all stages of production and before release of the final product, and careful documentation of each lot of product. So too, the production of plasma proteins is neither trivial nor fully automated. Hence, the assurance of safety afforded by current Good Manufacturing Practices may be compromised by human error that escapes audits by the manufacturer and/or the Food and Drug Administration. Increasing the number of production batches to counterbalance a smaller scale would of necessity increase the number of operations involved in manufacture and quality control and could increase the probability of human error.

Compared to other measures, such as donor screening and deferral and virus inactivation or removal during processing, restricting the manufacturing scale would be a relatively ineffective measure for improving safety. Nevertheless, screening is effective only for infectious agents that can be and are screened for; methods of inactivation are effective only against infectious agents that are susceptible to them. For example, there is legitimate concern about the causative agent of Creutzfeldt-Jakob disease, despite the theoretical nature of the risk of transmission by transfusion, because it cannot currently be detected in plasma and the extent to which it is inactivated or removed by current manufacturing methods is not known.[24,25] There is also a prevailing apprehension about infectious agents that might emerge in the blood supply at some future time. Such agents may resist the chemical or physical methods now employed to inactivate viruses and, at least initially, may be unrecognized and therefore undetectable. Should either danger materialize, experience has demonstrated that persons who depend on the continued receipt of plasma derivatives, such as hemophiliacs, would be highly vulnerable.[5,6,26] In this setting, the admittedly marginal benefit of restricting the size of plasma pools is attractive, because it is independent of the nature of or any knowledge of the infectious agent. Finally, the use of smaller plasma pools would lessen the impact of product withdrawal when the inclusion of a contaminated unit in a manufacturing pool is suspected. For example, the diagnosis of several frequent plasma donors with Creutzfeldt-Jakob disease has led on each occasion to large recalls of products.[25]

The question of limiting pool size may deserve further consideration and more detailed analyses. It may be possible to achieve similar benefits by limiting certain practices that have a small impact on cost or supply (e.g., not pooling intermediate batches into larger fill lots unless absolutely necessary). The number of donors to whom the recipients of plasma derivatives are exposed might also be reduced by restructuring the patterns of distribution of these products. For instance, the distribution of each lot of a given product could be restricted to specific geographic areas. If all lots distributed to any one area were manufactured from plasma pools derived from a specific subpopulation of donors, the recipients of that product would therefore be exposed to plasma from fewer donors. To be fully effective, such a program would have to maintain the relationship between a small number of donors and a small number of patients over a significant period. It is not known whether such a program is feasible, and, in any event, substantial logistical problems would have to be overcome.

However, it is likely that greater safety margins can be achieved by a better scientific understanding of those infectious agents that are still transmitted by transfusion; by improved, and perhaps broader, screening programs; by the development of more effective virus inactivation and/or removal techniques; and by continued, vigilant surveillance to detect the emergence of new pathogens at the earliest possible time.

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Literature Reference 4

Hepatitis C Virus Infection Associated With Administration of Intravenous Immune Globulin

A Cohort Study

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Objective.—To determine the risk of and risk factors for hepatitis C virus (HCV) infection among persons with immune deficiencies who had received intravenous immune globulin (IGIV) between March 1993 and February 1994.

Design.—Retrospective cohort study.

Setting.—An immunology program in a tertiary care hospital.

Patients.—Of 341 persons who had received IGIV between March 1, 1993, and February 22, 1994, 278 (82%) were enrolled. The mean age for the enrolled persons was 9 years, and 99% had primary immune deficiencies.

Main Outcome Measures.—Evidence of HCV infection by detection in sera of antibody to HCV and/or HCV RNA by reverse transcriptase polymerase chain reaction.

Results.—Twenty-three (11%) of 210 persons who received the IGIV Gammagard (Baxter Healthcare Corporation, Deerfield, Ill) became infected compared with none of 52 persons who received exclusively other IGIV products ($P=.01$). In a multivariate analysis, HCV infection was associated only with Gammagard produced from plasma screened by second-generation (multiantigen) anti-HCV tests ($P=.03$). Hepatitis C virus RNA was detected in Gammagard, and the risk of transmission to recipients increased with increasing quantity of HCV RNA infused, from 0 for those who received no HCV RNA-positive lots to 29% for the quartile of patients receiving the greatest amount ($P<.001$). At least 9 different lots of Gammagard were required to account for all cases.

Conclusion.—Gammagard was the only IGIV product implicated in the transmission of HCV. Infection was associated with higher quantities of HCV RNA in Gammagard produced from second-generation anti-HCV–screened plasma. Further studies are needed to determine reasons for the infectivity of Gammagard, and viral inactivation and removal steps are needed to ensure the safety of IGIV products.

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INTRAVENOUS immune globulin (IGIV) preparations are human plasma–derived products used for antibody replacement in persons with immunodeficiency disorders and for treatment of persons with immune-mediated diseases. Intravenous immune globulin distributed in the United States is produced by cold ethanol fractionation of large plasma pools and has been used safely in clinical settings since the early 1980s. Beginning in October 1991, plasma used to make IGIV was screened using single-antigen (first-generation) tests for antibody to hepatitis C virus (anti-HCV), and anti-HCV–positive units of plasma were discarded. More sensitive multiantigen (second-generation) tests were licensed in March 1992, and IGIV was produced using plasma donations screened using these tests by November 1992. Intravenous immune globulin manufactured exclusively from second-generation screened plasma was first distributed in early 1993.

Several outbreaks of non-A, non-B hepatitis associated with IGIV administration have been reported.[1-6] All but one of these outbreaks were associated with preparations of IGIV that were manufactured in Europe; one was associated with a prelicensure US product. Retrospective analysis of these outbreaks confirmed these non-A, non-B hepatitis cases as HCV infections.[7] Immune globulin products (IGIV and intramuscular immune globulin) licensed in the United States have not been previously associated with transmission of bloodborne agents, including HCV.

On February 23, 1994, Baxter Healthcare Corporation (BHC), Deerfield, Ill, issued a voluntary recall of its 2 IGIV preparations, Gammagard and Polygam, in response to reports of acute hepatitis C among persons who had received Gammagard. As of January 1996, 134 cases of HCV infection among persons who had received Gammagard had been reported to the Centers for Disease Control and Prevention. As a result of these case reports, we initiated an investigation to determine the risk and risk factors for HCV infection among persons who had received IGIV products in the United States.[8] The results of a cohort study involving patients with immune deficiencies who received Gammagard and other manufacturers' IGIV products at a large immunology center are presented in this article.

METHODS

Patients

All persons followed in the Allergy/Clinical Immunology Program at Children's Hospital in Boston, Mass, who received IGIV between March 1, 1993, and February 22, 1994 (study period) were eligible for the study. Persons with chronic hepatitis as determined by medical history were excluded. Children's Hospital is a large pediatric hospital with approximately 16 000 admissions per year, and the Allergy/Clinical Immunology Program provides treatment for approximately 400 children and adults with primary immune deficiencies. This site was chosen because of the large number of persons receiving IGIV, the availability of accurate data on IGIV infusions, and because, following the recall of Gammagard, several persons receiving care at the clinic were found to have evidence of acute HCV infection. The study was approved by institutional review boards at Children's Hospital and at the Centers for Disease Control and Prevention. Written informed consent to participate in the study was obtained from each patient or parent.

Data Collection

For each eligible patient, the medical record was reviewed to extract demographic information; pertinent medical history; the indication for receipt of IGIV; and dates, dosages, brands, and, if available, lot numbers of IGIV administered during the study period. Known risk factors for HCV infection were assessed by review of medical records and by a questionnaire completed by each patient or his or her parent. For persons with HCV infection, data regarding the acute illness, including date of onset, presence of signs and/or symptoms, and liver function test results, were collected using a questionnaire completed by the case patient's treating physician.

At least 1 serum specimen was obtained from each participant between March 1, 1994, and February 28, 1995. All sera were tested for alanine aminotransferase (ALT) levels within 24 hours of collection. Additional sera were frozen within 4 hours of collection and stored at −70°C. These specimens were tested for anti-HCV by a second-generation enzyme immunoassay (EIA) (HCV EIA 2.0, Abbott Laboratories, North Chicago, Ill) at either the Hepatitis Branch, Centers for Disease Control and Prevention, or a laboratory using the same methods. Specimens repeatedly reactive by EIA were tested by a supplemental assay (MATRIX HCV, Abbott Laboratories, Abbott Park, Ill). Sera testing positive by both EIA and MATRIX were considered anti-HCV positive. Reverse transcriptase–polymerase chain reaction (RT-PCR) testing for HCV RNA (modified Amplicor system, Roche Laboratories, Nutley, NJ) was performed by an independent reference laboratory (Metpath, Teterboro, NJ) on serum specimens from 180 patients, including all persons with ALT levels 2.5 or more times the upper limit of normal (ULN, 30 U/L) and on sera from a sample of patients with normal or mildly elevated ALT levels (<2.5 times ULN). Sera from case patients were also tested for hepatitis B surface antigen (AUSRIA, Abbott Laboratories), IgM antibody to hepatitis B core antigen (Corzyme-M, Abbott Laboratories), and IgM antibody to hepatitis A virus (HAVAB-M, Abbott Laboratories).

A list of lots and dates of manufacture of Gammagard distributed in the United States during 1993 was obtained from BHC; for some lots, the relative proportions of unscreened plasma and plasma screened by first-generation and second-generation anti-HCV tests used to produce the lot were obtained. Lots for which these data were not available and were produced after April 10, 1993, were considered to have been produced solely using second-generation screened plasma, whereas lots produced before this date were considered to have been produced from first-generation screened plasma. This assumption is supported by published data[3] and information provided by BHC.

Lots of Gammagard that were administered to at least 10 persons in the cohort were tested at the Food and Drug Administration for the presence of HCV RNA by RT-PCR, and the titer of HCV RNA in positive lots was determined by quantitative RT-PCR testing. To perform RT-PCR testing, Gammagard was reconstituted to 10% immunoglobulin G (IgG) with phosphate-buffered saline (pH, 7.4). A 4-mL aliquot of each reconstituted solution was incubated with 0.4 mL of proteinase K (15.5 mg/mL, Boehringer Mannheim, Indianapolis, Ind), 0.5 mL of 20% sodium dodecyl sulfate, and 0.1 mL of TEN buffer (50 mmol/L Tris hydrochloride [pH 8.0], 1 mmol/L sodium ethylenediaminetetraacetic acid, 150 mmol/L sodium chloride) at 60°C for 1 hour. Then, 0.5 mL of 2 mol/L sodium acetate buffer (pH 4.0), 5 mL of buffered saturated phenol (Gibco BRL, Grand Island, NY), and 2.5 mL of chloroform/isoamylalcohol (24:1) were added and mixed thoroughly. The rest of the RNA extraction procedure has been previously described.[4] The resulting RNA was dissolved in 100 μL of diethyl pyrocarbonate–treated water supplemented with 40 U of recombinant ribonuclease inhibitor (Amersham, Arlington Heights, Ill) and was 10^{24}-fold serially diluted. Fifty-milliliter aliquots of each dilution were used for RT-PCR as described before.[3] One PCR unit was arbitrarily defined as the minimal quantity of HCV RNA from which an amplified product could be visualized by ethidium bromide staining. The amount of HCV RNA (in PCR units) per replicate volume was calculated accordingly, and the results were expressed as units per gram of IgG.

Case Definition

A "probable" case of acute HCV infection was defined as (1) an ALT level of 2.5 or more times the ULN with a previously documented normal ALT level, or a single ALT level 6 or more times the ULN, and (2) a positive anti-HCV test and/or detectable HCV RNA by PCR. A "possible" case was defined as (1) an ALT level of less than 2.5 times the ULN, and (2) a positive anti-HCV test and/or detectable HCV RNA by PCR.

Data Analysis

Statistical differences between groups were evaluated by χ^2 and Fisher exact test for dichotomous variables and by the Student t test or Wilcoxon rank sum test for continuous variables. P values less than .05 were considered to be statistically significant. Multivariate analyses were performed by logistic regression. For each participant, the amount of HCV RNA received during the study period was calculated by multiplying the units of HCV RNA per gram in each particular lot of Gammagard by the number of grams received by the patient from that lot, and then adding the units from all lots from which the patient received product.

RESULTS

Characteristics of Study Population

Of 341 eligible persons, 278 (82%) were enrolled; the median age of persons enrolled was 9 years (range, 10 months to 65 years), and 160 (58%) were male. Treatment of primary immune deficiency was the indication for IGIV therapy for 275 persons (99%), including common variable immunodeficiency, 195; IgG subclass deficiency, 48; X-linked agammaglobulinemia, 13; transient hypogammaglobulinemia of infancy, 7; ataxia-telangiectasia, 3; X-linked hyper-IgM syndrome, 3; severe combined immunodeficiency, 3; hyper-IgE syndrome, 2; and DiGeorge syndrome, 1. Three persons received IGIV for treatment of immune-mediated diseases (pulmonary hemosiderosis, central nervous system

Table 1.—Alanine Aminotransferase (ALT) Levels and Presence of Antibody to Hepatitis C Virus (Anti-HCV) and HCV RNA Among Intravenous Immune Globulin (IGIV) Recipients

ALT Level*	Anti-HCV	No.	No. HCV RNA Positive/No. Tested (%)
≥2.5	Positive	22	21/22 (95)
	Negative	7	1/7 (14)
1.1-2.4	Positive	2	0/2 (0)
	Negative	34	1/20 (5)
≤1.0	Positive	2	0/2 (0)
	Negative	211	1/127 (1)
Total	Positive	26	21/26 (81)
	Negative	252	3/154 (3)

*Values are multiples of the upper limit of normal for ALT.

Table 2.—Incidence of Hepatitis C Virus (HCV) Infection Among Intravenous Immune Globulin (IGIV) Recipients by Demographic Variables and Exposure to Brand and Dose of IGIV

Variable	Total No. of Patients	HCV Infection, No. (%)	Relative Risk	95% Confidence Interval	P
Age, y					
<9	130	4 (3)	0.23	0.1-0.7	.005
≥9	142	19 (13)	...	...	
Sex					
Male	157	15 (10)	1.37	0.6-3.1	.59
Female	115	8 (7)	...	...	
Risk factors for HCV infection					
Yes	15	1 (7)	0.78	0.1-5.4	1.00
No	257	22 (9)	...	...	
Brand(s) received*					
Gammagard					
Yes	210	23 (11)	Unrefined	Undefined	.01
No	52	0 (0)	...	...	
Polygam					
Yes	129	10 (8)	0.79	0.4-1.7	.72
No	133	13 (10)	...	...	
Iveegam					
Yes	147	10 (7)	0.60	0.3-1.3	.29
No	115	13 (11)	...	...	
Gammune-N					
Yes	46	1 (2)	0.21	0.03-1.5	.09
No	216	22 (10)	...	...	
Dose of Gammagard, g†					
0	52	0 (0)	...	...	.001‡
1-40	56	0 (0)	...	...	
41-114	49	3 (6)	...	...	
115-264	53	6 (11)	...	...	
≥265	52	14 (27)	...	...	

*Brand and dose of IGIV were available for 262 of the 272 persons included in the analyses (Gammagard and Polygam, Baxter Healthcare Corp, Deerfield, Ill; Iveegam, ÖTH, Vienna, Austria; and Gammune-N, Bayer Corp, Berkeley, Calif).
†Categories greater than 0 represent quartiles of the doses received by the cohort.
‡χ^2 for trend.

vasculitis, and immune thrombocytopenic purpura). Persons enrolled were younger than persons not enrolled (median age, 9 vs 13 years; $P=.003$) and were more likely to have received Gammagard (80% vs 50%; $P<.001$). The groups were similar with respect to sex, indication for IGIV, and dose of IGIV received.

Laboratory Testing

Of the 278 persons enrolled, 65 (23%) had ALT levels greater than the ULN on at least 1 test; 29 (10%) had ALT levels 2.5 or more times the ULN (Table 1). Anti-HCV was detected in 26 persons (9%), including 22 (76%) of the 29 who had ALT levels more than 2.5 times the ULN, 2 (6%) of 36 with ALT levels 1.1 to 2.5 times the ULN, and 2 (1%) of 213 with normal ALT levels.

Of the 26 persons who were anti-HCV positive, 21 (81%) had detectable HCV RNA by PCR compared with 3 (2%) of the 154 persons who tested negative for anti-HCV (Table 1). Of these 3, 2 had X-linked agammaglobulinemia and 1 had common variable immunodeficiency with reduced T-cell function and no humoral response to specific antigens on previous testing.

Characteristics of Case Patients

Twenty-nine (10%) of 278 persons had evidence of HCV infection (anti-HCV and/or HCV RNA positive); of these, 23 (79%) had probable cases of acute HCV infection and 6 had possible cases. Only the 23 persons with probable cases and the 249 noninfected persons are included in the analyses presented here, although inclusion of the 6 persons with possible cases did not alter any of the associations detected. By univariate analysis, older age was associated with increased risk for HCV infection, whereas sex and the presence of known risk factors for HCV infection were not associated with infection (Table 2). Of IGIV recipients with known risk factors for HCV infection, one was a case patient who had received transfusions with blood products other than IGIV, and 14 were noncase patients (10 had received transfusions and 4 were employed as health care workers). Seventeen (74%) of 23 probable case patients reported signs and/or symptoms of hepatitis, with dates of illness onset from March to June 1994. None of the case patients had serologic evidence of acute hepatitis A virus or hepatitis B virus infection, and none had evidence of other causes of hepatitis.

Relationship of Dose and Brand to HCV Infection

Fifty (19%) of 262 persons received only Gammagard, 160 (61%) received Gammagard in addition to other brands of IGIV, and 52 (20%) exclusively received non-Gammagard products (the brand of IGIV infusion could not be determined for 10 persons). Hepatitis C virus infection was associated with receipt of Gammagard, but not with receipt of other IGIV products (Table 2). The attack rate was 11% (23/210) among all persons who received Gammagard, and 14% (7/50) among persons who exclusively received Gammagard. Moreover, the risk of HCV infection increased with receipt of larger doses of Gammagard (Table 2). After adjusting for age, receipt of larger doses remained a significant risk factor ($P=.01$). All 6 persons with possible cases also received Gammagard. No cases were detected among persons who did not receive Gammagard.

Relationship of Specific Lots to HCV Infection

Lot numbers administered were available for 201 (96%) of the 210 persons who received Gammagard. Patients received Gammagard from a median of 7 different lots (range, 1-31). A minimum of 9 separate lots was required to account for all 23 probable cases. Ninety-one different lots of Gammagard were administered to the cohort during the study period; 48 (53%) were produced from plasma screened with second generation anti-HCV tests, and 43 (47%) were produced from unscreened plasma or plasma screened with first-generation anti-HCV tests. The lot-specific attack rates (number of case patients who received the lot divided by total number of persons who received the lot) for all lots administered to the cohort produced with second-generation screened plasma (200/1191 [16.8%]) were significantly higher than rates for lots produced with

unscreened plasma or first generation screened plasma (57/564 [10.1%], $P<.001$). In addition, by univariate analysis, the risk of HCV infection increased significantly with receipt of higher doses of Gammagard produced from second-generation anti-HCV-screened plasma, and with Gammagard produced from unscreened and first-generation screened plasma (Table 3). None of the 8 persons who received Gammagard exclusively made from unscreened or first-generation screened plasma became infected.

Gammagard lots produced from plasma screened with second-generation anti-HCV tests were more likely to be HCV RNA positive than lots produced with unscreened or first generation anti-HCV-screened plasma (21/36 vs 0/9, $P<.002$). Receipt of IGIV from lots positive for HCV RNA was significantly associated with infection (Table 3). The attack rate increased with increasing number of HCV RNA-positive infusions received and with increasing amounts of HCV RNA infused. No HCV infections were detected among 37 persons who received Gammagard, but who had received only HCV RNA-negative lots.

Variables significantly associated with the risk of acquiring HCV infection by univariate analyses were analyzed by multiple logistic regression. Analyses were performed using continuous variables except for dose of first- and second-generation Gammagard, which were analyzed as categorical variables; for these, the data were split by median dose into a high- and low-dose category. In these analyses, the quantity of HCV RNA received (odds ratio [OR]=1.07 per 1000 PCR units; 95% confidence interval [CI], 1.04-1.09; $P<.01$) and receiving a high dose of Gammagard produced from second-generation anti-HCV-screened plasma (OR=5.3; 95% CI, 1.1-24.8; $P=.04$) were independently associated with acquiring HCV infection, whereas age (OR=1.0; 95% CI, 0.97-1.04), the number of HCV RNA-positive Gammagard lots received (OR=0.9; 95% CI, 0.8-1.1), and dose of Gammagard produced from unscreened or first-generation anti-HCV-screened plasma (OR=1.5; 95% CI, 0.5-4.6) were not associated with infection.

Table 3.—Incidence of Hepatitis C Virus (HCV) Infection Among Gammagard Recipients by Exposure to HCV RNA–Positive Lots, Quantity of HCV RNA Infused, and Dose of Gammagard Made From Plasma Screened by Second-Generation Anti-HCV Tests

Variable	Total No. of Persons	HCV Infection, No. (%)	P
Receipt of HCV RNA–positive lots			
Yes	164	23 (14)	.01
No	37	0 (0)	
No. of infusions of HCV RNA–positive lots			
None	37	0 (0)	.001‡
1-4	83	7 (8)	
≥5	81	16 (20)	
Quantity of HCV RNA infused, U*†			
0	37	0 (0)	.001‡
1-1089	40	1 (3)	
1090-3269	40	6 (15)	
3270-8509	42	4 (10)	
≥8510	41	12 (29)	
Dose of Gammagard made from unscreened or first-generation anti-HCV–screened plasma, g†			
0-3	46	4 (8)	.03‡
4-20	45	2 (4)	
21-65	57	7 (12)	
>65	51	10 (20)	
Dose of Gammagard made from second-generation anti-HCV–screened plasma, g†			
0	8	0 (0)	.002‡
1-29	45	0 (0)	
30-64	49	3 (6)	
65-174	50	8 (15)	
≥175	49	12 (24)	

*See "Methods."

†Categories greater than 0 represent quartiles of the doses received by the cohort. The quantity of HCV RNA–positive Gammagard received by 1 participant could not be calculated, as the HCV RNA–positive lot received was not tested by quantitative polymerase chain reaction.

‡χ^2 for trend.

COMMENT

This is the first occurrence of HCV transmission associated with a commercially licensed immune globulin product distributed in the United States. Among persons in this cohort who received Gammagard, 11% became infected with HCV; no HCV infections were found among persons who received exclusively other IGIV products. The risk of acquiring infection was significantly associated with the dose received of Gammagard produced from plasma screened with second-generation anti-HCV tests. In addition, all cases of HCV infection were among persons who received Gammagard from at least 1 lot that tested positive for HCV RNA, and there was a dose-response relationship between HCV infection and the quantity of HCV RNA infused.

The dose-response relationship between receipt of higher doses of Gammagard produced from plasma screened with second-generation anti-HCV tests and HCV infection supports the hypothesis that screening of plasma with second-generation anti-HCV tests adversely affected the safety of the product. Despite initial concerns about the safety of IGIV made with anti-HCV screened plasma,[10] the use of first-generation anti-HCV tests for screening of donors was shown to reduce the concentration of HCV by 83% in a plasma pool composed solely of anti-HCV–negative donations compared with an unscreened plasma pool.[11] In addition, an experimental study in chimpanzees demonstrated that IGIV manufactured from first-generation anti-HCV–screened plasma did not transmit HCV.[12] However, in contrast to screening with first-generation tests, screening with second-generation anti-HCV tests may have removed virtually all complexing antibodies from plasma used to make IGIV, compromising the ability of the manufacturing process to partition HCV into non-immunoglobulin fractions,[13] and resulting in a higher proportion of HCV in the immunoglobulin fraction.

Why was HCV infection only associated with receipt of Gammagard when all manufacturers of IGIV products instituted screening of plasma with second-generation anti-HCV tests? Gammagard is 1 of 8 IGIV products that did not include a viral inactivation step in the manufacturing process during the study period. The absence of a viral inactivation step in the manufacturing process of Gammagard may have left the process unable to eliminate infectious virus. Other differences in production methods between Gammagard and other IGIV products, such as subtle modifications in the Cohn-Oncley fractionation process, differences in viral distribution during the additional processing steps, and differences in donor populations may explain the occurrence of HCV infection among recipients of IGIV from a single manufacturer. Since multiple lots manufactured over a several-month time pe-

riod would have to have been contaminated to explain the occurrence of all cases in the cohort studied in this article, a single manufacturing error is an unlikely explanation for the outbreak.

In June 1994, the US Public Health Service recommended that persons who received Gammagard after April 1, 1993, be screened for HCV infection with tests for ALT and anti-HCV. Screening was also recommended for persons who had received another IGIV product manufactured by BHC, Polygam, which is produced using identical methods, but from a different plasma source (ie, volunteer b[1] od donors). The reported sensitivity of second-generation EIAs for anti-HCV among immune-competent persons is about 90%, and most of these persons become anti-HCV positive within 6 months of infection.[14-16] However, because of concerns that anti-HCV would not be detectable in persons who were immunocompromised,[17-20] and because the timing of anti-HCV response among immunocompromised persons was unknown, PCR testing for HCV RNA was recommended for persons with elevated ALT levels but who were anti-HCV negative on repeated testing. The use of ALT and anti-HCV tests as screening tests in IGIV recipients is supported by this study; ALT and anti-HCV screening was 96% sensitive in detecting HCV infection compared with PCR, and only 1 person with X-linked agammaglobulinemia and absent humoral responses who had normal ALT values and who lacked detectable anti-HCV had evidence of HCV RNA by PCR.

Despite conducting this study in a single site, the data should be generalizable to other populations since the risk of infection was not limited to specific lots, but was associated with the type of screening of plasma used in production. Furthermore, all brands of IGIV and more than 70% of all Gammagard lots distributed in the United States were administered to this cohort. Potential limitations of this study are the possibility for ascertainment and misclassification bias. The attack rate may be overestimated if persons with symptomatic HCV infection were more likely to be screened, or if persons with chronic HCV infection were included as case patients. However, if all nonparticipants were uninfected, the rate of acute HCV infection among Gammagard users would still be 9%. Misclassification of chronically infected persons is unlikely because of the exclusion of case patients without marked ALT elevations (who would be more likely to have chronic disease), the lack of other risk factors for HCV infection among the case patients, and the strong temporal association with IGIV produced since April 1993. Because of the unavailability of some lot-specific distribution data, we could not estimate the total number of persons infected in the United States. However, given the high attack rates in this cohort, we would expect that the number of case patients in the United States ascertained primarily through passive reporting represents an underestimate.

This outbreak serves as a reminder of the possible risks associated with the administration of blood products, even though multiple steps are taken to decrease the potential for transmission of viruses by these products. As the Food and Drug Administration has requested, all US-licensed IGIV products now have viral inactivation steps included in the manufacturing process as a method to further reduce the likelihood of viral transmission. Additionally, all immunoglobulin products (including intramuscular immunoglobulins, which have not been associated with hepatitis C) that lack a viral inactivation step(s) are now screened by PCR tests for HCV RNA, and only product that is HCV RNA negative is released for dist[-]bution. In this study, no persons who received only HCV RNA–negative material became infected. Although the presence of HCV RNA in immune globulin products may not necessarily correlate with infectivity, these safeguards should help prevent future transmission of HCV by these products.

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Literature Reference 5

Inactivation and partition of human T-cell lymphotrophic virus, type III, during ethanol fractionation of plasma

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ABSTRACT: Because of concern about the safety of immune globulins prepared for injection, we studied the effects of ethanol fractionation of human plasma on human lymphotropic virus, type III, (HTLV-III) by spiking the products of various fractionation steps with HTLV-III. Tests of inactivation and removal indicated that the ratio of residual live virus in plasma fractions/live virus in starting plasma was about 1×10^{-15} for precipitate II from which immune globulin for injection is manufactured. The results are reassuring regarding the potential safety of immune globulin.

The virus which causes the acquired immunodeficiency syndrome (AIDS), variably designated human T-cell lymphotrophic virus, type III (HTLV-III), lymphadenopathy-associated virus (LAV) and AIDS-associated retrovirus (ARV) is transmitted by parenteral exposure to contaminated blood (1-3) and clotting factor concentrates (4-6). Although there have been no reported cases of AIDS resulting from receipt of immune globulin products, concern about their safety has been expressed, especially for immune globulin administered intravenously. Some studies of HTLV-III inactivation have been reported using heat, ultraviolet and gamma irradiation and various chemicals (7-13). Inactivation of HTLV-III by alcohol is particularly relevant to the safety of immune globulin (10,11,13). A comprehensive evaluation of the capacity of the Cohn Oncley alcohol fractionation process to remove infectious HTLV-III has not been published. We report here the results of such studies. We studied the kinetics of inactivation of HTLV-III by varying concentrations of ethanol in different plasma fractions. We also studied the partitioning and inactivation of HTLV-III during each step in the production of immune globulin.

Methods and Materials

Virus and cell lines

The human T leukemia cell line (H9 cells), and the chronically infected HTLV-III-producing III-B clone of this cell line were provided by Dr. Robert Gallo (National Cancer Institute, Bethesda, MD) (1,14-15). They were maintained in culture in RPMI 1640 medium supplemented with 20 percent fetal bovine serum (FBS), penicillin, streptomycin and glutamine. Medium was harvested periodically from the III B cell cultures for use as virus inocula. Virus was concentrated from these cell culture fluids by precipitation with polyethylene glycol (PEG, Carbowax 8000, Fisher Scientific, Pittsburgh, PA). A 30 percent (w/v) mixture of PEG in cell culture fluid was placed at 4°C for 2 h, centrifuged at 1100 x g for 30 min at 4°C, then resuspended at 1/10 or 1/100 the original volume. This process consistently produced a concentrated virus without loss of infectivity in our assays.

Viral infectivity assays

The H9 cell line was used for infectivity assays. Hexadimethrine bromide (Polybrene, Sigma Chemical, St. Louis, MO) at 2 ug per ml was added for 1 h prior to virus inoculation. Virus suspensions were tested in duplicate or triplicate serial ten-fold dilutions prepared in medium with 2 ug per ml Polybrene and added to cell suspensions of 5×10^5 cells in a total volume of 1 ml. Aliquots of each virus dilution were allowed to adsorb for 90 min, washed once, and medium was added to give final cell concentrations of 2.5×10^5 cells per ml. The suspensions were dispensed in 2-ml aliquots into wells of 24-well plastic tissue culture plates (Falcon, Oxnard, CA), covered, placed in plastic bags and incubated at 37°C in a 5 percent CO2-in-air humidified atmosphere. The plates were maintained in medium containing 1 ug per ml of Polybrene for 4 to 6 weeks and sampled at intervals throughout. Results of infectivity assays were based on observations for cytoplasmic antigen expression detected by immunofluorescence assay (IFA), and reverse transcriptase (RT) and antigen-capture enzyme-linked immunosorbent assays (ELISA) of cell culture fluids. Cultures were routinely monitored by two or three of the methods, and results obtained by the different methods were always concordant within one dilution. The highest dilution producing positive results was considered to contain one in vitro infectious unit (IVIU). RT assays were performed as described by Poiesz, et al. (16). The IFA tests involved fixation of cells in cold acetone for 10 min, and use of a human anti-HTLV-III immune globulin preparation and a goat anti-human globulin conjugated to fluorescein isothyocyanate. Moderately intense fluorescence involving at least 5 percent of cells was considered a positive reaction.

The antigen-capture fluorescent ELISA for HTLV-III was performed on microtiter plates (Dynatech Laboratories, Alexandria, VA) coated with human IgG derived from a patient with AIDS. Sample culture supernatants, as well as control HTLV-III infected and uninfected H9 cell culture supernatants, diluted in equal volumes of 1 percent Nonidet P40 (Sigma Chemical Co., St. Louis, MO) in 1 M NaCl were added to wells and incubated overnight at 37°C. Antigen detection involved sequential 1-h incubations of the plates with serum from a rabbit immunized with HTLV-III core protein (p24), followed by alkaline phosphatase-conjugated goat anti-rabbit IgG (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Conversion of the fluorogenic substrate, 4-methylumbelliferyl phosphate (Sigma), was measured with an automated fluorescent

spectrophotometer (Microfluor 600, Dynatech). A reactivity index (RI) was calculated by dividing the sample fluorescence by that of the negative control after correcting each for the blank. Samples with an RI greater than 3.0 (approximately 99 percent upper confidence limit for negative controls) were considered positive for HTLV-III core antigen. In some experiments the test was used to quantitate relative amounts of antigen in various specimens. Virus concentration was proportional to RI (r^2 = 0.96, $p<.01$ in all such experiments). Relative amounts of antigen in specimens being compared were determined by comparison of the dilution curves.

Inactivation of HTLV-III by ethanol and removal by the Cohn-Oncley fractionation process

To study kinetics of inactivation of HTLV-III by ethanol, samples of cryoprecipitate-poor plasma, supernatant I, and supernatant III were adjusted with 95 percent ethanol to the desired alcohol concentrations, the mixtures brought to -5°C, and PEG-concentrated HTLV-III, prechilled in an ice-water bath, was added. The mixtures were held at -5°C for the desired time, diluted 1:20 with medium, the virus precipitated with PEG, and then suspended in medium for testing.

To test partitioning and inactivation of virus during formation of cryoprecipitate, HTLV-III was added to freshly-drawn citrated plasma. The mixture was placed at -20°C overnight, thawed slowly at 4°C, centrifuged at 16,000 x g for 15 min at 4°C, and the fractions separated for testing. The virus concentrates added to this and other fractions were prepared by precipitation with PEG from tissue culture medium. To test the effects of other steps in the production of immune globulin, virus was added at each step and partitioning and inactivation at each step were determined.

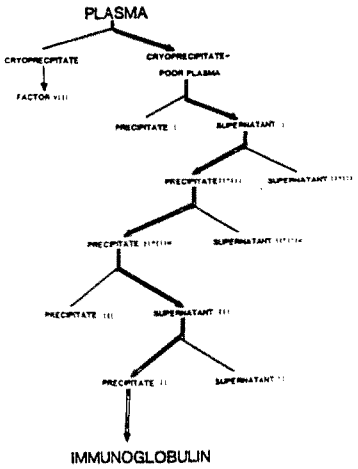

Fig. 1. Schematic diagram of Cohn-Oncley fractionation process studied for effects on HTLV-III infectivity (See text for methods).

Plasma fractionation was carried out under aseptic conditions by the methods of Cohn (17) and Oncley (18) which are summarized in Figure 1. A single unit of plasma made from blood drawn into citrate-phosphate-dextrose (pH 7.12), negative for antibody to HTLV-III, was cooled to 0°C and alcohol (SDA-3A; 90% ethanol, 5% methanol, 5% water) was added up to a concentration of 8 percent (all alcohol percentages are v/v) as the temperature was lowered to -3°C. The precipitate (fraction I) was removed by centrifugation (16,000 x g, 15 min, -3°C) and discarded. The supernatant I was adjusted to pH 6.75, and alcohol was added up to a concentration of 21 percent while lowering the temperature to -5°C. The precipitate II+III was collected by centrifugation (16,000 x g, 15 min, -5°C) and suspended in 12 volumes of ice water. This solution (pH 6.73) was then brought to 20 percent alcohol while cooling to -5°C. The precipitate II+IIIw was collected by centrifugation as above, redissolved in 17 volumes of ice water, adjusted to pH 5.39, and alcohol was added to 17 percent while cooling to -5°C. This mixture was centrifuged as above, precipitate III was discarded, and the supernatant III was adjusted to pH 7.27. These samples of plasma, supernatant I, suspension II+III, suspension II+IIIw, and supernatant III were brought to the appropriate temperature (0°C for plasma, suspension II+III, and suspension II+IIIw; -5°C for supernatant I and supernatant III), then 1/20 volume of PEG-concentrated virus, at 0°C, was added. These spiked samples of plasma, supernatant I, suspension II+III, suspension II+IIIw, and supernatant III were cooled to -5°C while alcohol was added to achieve final concentrations of 8, 21, 20, 17, and 25 percent, respectively. The supernatant III, at 25 percent ethanol, was kept at -5°C for 24 h, whereas the other samples were held only 2 h prior to centrifugation (16,000 x g, 15 min, -5°C). Each precipitate was resuspended directly in medium for testing; the supernatants were first diluted 1:20 in medium and precipitated with PEG (to remove alcohol), then resuspended in medium for testing.

Results

Inactivation of HTLV-III as a function of ethanol concentration and time

The effects of ethanol on HTLV-III infectivity in plasma fractions are shown in Table 1. After 30 min in 10 to 20 percent ethanol in cryoprecipitate-poor plasma, virus titer was reduced by 10^{-1} or 10^{-2}. After 30 min in 25 to 40 percent ethanol, titers were reduced to less than 1×10^{-4} times the starting titer. Similar results were obtained in a second experiment of this type. In supernatant I and supernatant III inactivation occurred more slowly than in cryoprecipitate poor plasma. Residual infectivity was detected in these supernatants after 24 to 48 h of exposure to ethanol. In additional experiments (not shown), inactivation of HTLV-III in unprocessed plasma by 25 percent ethanol occurred at a rate of more than 10^5 IVIU per ml in 30 min.

Table 1. Inactivation of HTLV-III added to plasma fractions as a function of ethanol concentration and time

Plasma Fraction Tested	Ethanol Concentration %	Time of Exposure	Infectivity IVIU/ml
Cryo-poor Plasma	0	30 min	10^4
	10	"	10^3
	20	"	10^2
	25	"	$<10^0$
	30	"	$<10^0$
	40	"	$<10^0$
Cryo-poor Plasma	25	0	10^4
		10 min	10^2
		30 min	$<10^0$
		1 h	$<10^0$
		2 h	$<10^0$
Supernatant I	25	0	10^4
		2 h	10^3
		24 h	10^0
		48 h	10^1
Supernatant III	25	0	10^3
		2 h	10^3
		4 h	10^2
		24 h	10^0

Partitioning of HTLV-III during plasma fractionation

To evaluate the efficacy of the Cohn Oncley process in removing or inactivating HTLV-III, virus was added to various fractions prior to precipitation, as summarized in Table 2. The titer of virus in the plasma used to form the cryoprecipitate, after addition of PEG concentrated HTLV-III, was greater than or equal to 1×10^6 IVIU per ml. Aliquots of a different concentrated virus preparation were added to equal volumes of each of the other fractions to be processed. The geometric mean titer ± SD of these other fractions immediately after addition of this virus pool was 4.0 ± 0.5 $\log_{10}$ IVIU per ml. The infectivity titrations of the individual fractions produced in the experiments shown in Table 2 yielded identical results in the duplicate tests. It was found by ELISA that more antigen partitioned into precipitates than supernatants. There were varying degrees of reduction in infectivity at each step compared to the starting titer of the spiked plasma fractions (1×10^6 IVIU/ml). These reductions were due in part to partitioning and in part to inactivation. The results shown in Table 2 are similar to those obtained in additional experiments involving spiking of plasma, cryoprecipitate-poor plasma, supernatant I, and precipitate II+IIIw. In the repeated experiment to examine fractionation of plasma, the infectivity of cryoprecipitate formed was one-tenth of the infectivity of the plasma from which it was obtained, while the relative titers in the other fractions were similar to those shown in Table 2.

Discussion

Ethanol appeared to be more effective for inactivating HTLV-III in plasma or cryoprecipitate-poor plasma than in plasma fractions from subsequent steps in the Cohn-Oncley fractionation process. Partitioning and/or inactivation of virus occurred at most steps in the process. These results provide a basis for assessing the potential safety of immune globulin fractions for human administration.

Immune globulins are produced by methods generally similar to that represented in Figure 1. Validation of specific manufacturing processes for effectiveness in elimination of HTLV-III will be of value. The efficiency of each of the six steps we studied in removing virus ranged from 10^1 to more than 10^4 IVIU per ml. If the assumption is made that the efficiency of each step is independent of

Table 2. Inactivation and partitioning of HTLV-III during the Cohn-Oncley fractionation process

Starting Suspension	Fractions Produced: Plasma Fraction	Infectivity IVIU/ml	Antigen Content % of Total†
Plasma	Cryoprecipitate	$\geq 10^6$*	95
	Cryo-poor Plasma	10^3	5
Frozen-thawed Plasma	Precipitate I	10^4	65
	Supernatant I	10^3	35
Supernatant I	Precipitate II+III	10^1	95
	Supernatant II+III	$<10^0$	5
Suspension of Precipitate II+III	Precipitate II+IIIw	10^3	99
	Supernatant II+IIIw	10^0	1
Suspension of Precipitate II+IIIw	Precipitate III	10^4	99.5
	Supernatant III	$<10^0$	0.5
Supernatant III	Precipitate II	10^1	99.6
	Supernatant II	$<10^0$	0.4

*Infectious virus was detected in the most dilute specimen tested.
†Relative reactivity in ELISA of antigen in precipitates (P) and supernatants (S) was determined. Antigen percent in precipitate was calculated as $[(P/S)/(1 + P/S)] \times 100$. Antigen percent in supernatant was calculated as 100 - percent of antigen in precipitate. The data are consistent with recovery in P+S of 90% of spiked antigen.

the starting titer at the beginning of the step, the cumulative efficiency of the entire process is calculated to be such that infectivity is reduced by more than 1 x 10^{15} IVIU per ml. The results of our studies are consistent with other published data (10,12,13) indicating sensitivity to ethanol.

Routine screening of blood and plasma donors in the United States indicates that 0.17 to 0.3 percent of donors have antibodies repeatably reactive in HTLV-III ELISA screening tests (19,20). Approximately 3.8 per each 10,000 blood donors tested by the American Red Cross have antibodies to HTLV-III based on Western blot analysis (personal communication, Dr. Roger Lodd). Current screening tests are retimated to be 98.3 to 99.8 percent sensitive for detecting antibodies in sera from HTLV-III-infected persons. Our own studies indicate that the geometric mean infectivity titer of plasma from 43 HTLV-III infected patients was 0.02 IVIU per ml (Quinnan, unpublished data). These studies included specimens from nine plasma donors and eleven healthy homosexual men with antibodies to HTLV-III, nine homosexual men with chronic lymphadenopathy syndrome, and fourteen patients with AIDS. If it is assumed that the infectivity of plasma obtained from HTLV-III-infected blood and plasma donors is similar and the rates of infection are as summarized above, it can be estimated that a 1000-liter plasma pool would contain about 7.6 IVIU if prepared from unscreened donors and less than or equal to 0.13 IVIU if prepared from screened donors. These estimates might be too low if the plasma specimens we tested were less infectious than those of typical HTLV-III-infected blood donors or if HTLV-III in plasma is more infectious for humans than for cell cultures. For example, there is evidence that plasma from infected people who do not yet have antibodies to HTLV-III may have relatively high concentrations of virus (personal communication, G. Wyss). Even if the infectivity of plasma is higher than these estimates, the efficiency of the process we studied in removing virus far exceeds that needed to assume lack of infectivity of immune globulin preparations for injection.

Acknowledgements

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respiratory status is usually regained in a few months. Respiratory complications are slightly but not significantly higher in bilateral internal-thoracic-artery bypass grafting. We do not select patients for internal-thoracic-artery bypass surgery on the basis of preoperative ventilatory function.

Unadjusted and adjusted statistical comparisons were made between patients who received an internal-thoracic-artery graft and those who received vein bypass grafts only. Since there were differences in preoperative clinical characteristics between these groups, statistical procedures that adjust for such differences were employed. The statistical method used to test survival differences between the internal-thoracic-artery group and the vein-graft group is similar in concept to a multiple-regression model, although the dependent variable is the time to death. As in any regression analysis, factors that explain a considerable amount of the variability in the dependent variable are entered into the regression model. In our series, a model was developed in which these factors were initially forced into the regression model; for example, age was entered into the model so that any difference in survival due to differences in age distribution between the two groups would be adjusted for by the model. Age, gender, severity of angina, extent of coronary atherosclerosis, left ventricular function, completeness of revascularization, and year of surgery were simultaneously forced into the model so that the association of these factors with survival would be taken into account. Then we tested whether any of the remaining differences in survival could be explained by the presence of an internal-thoracic-artery graft. Unadjusted comparisons were also presented to show that results differed between the groups even without complex statistical adjustments. Given the differences in long-term patency rates between the internal-thoracic-artery graft and the vein graft, a properly designed randomized trial would be impossible to conduct without bias and would not be ethically sound. Thus, an observational study with appropriate statistical control may be the most practical approach.

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AIDS, BLOOD TRANSFUSIONS, AND DIRECTED DONATIONS

To the Editor: Public and medical hysteria about the acquired immunodeficiency syndrome (AIDS) has contributed to an increasing reluctance on the part of patients to receive homologous (blood-bank donor) blood. This reluctance has led to increasing interest in autologous transfusions and in the process of "directed" donations, in which the patient directly solicits donations from family or friends.

Patients and, occasionally, their physicians think that directed donations must be safer than regular blood-bank donor blood in terms of disease transmission. After all, one expects that friends or family members would never harbor diseases or belong to a high-risk group and lie to the blood bank about it. This may be a risky assumption. The regular blood-bank donor who donates altruistically and anonymously (as far as the recipient is concerned) can truthfully answer all questions. On the other hand, the directed donor is under extreme pressure to donate, and fear that friends or relatives will learn of his or her unsuitability as a donor may easily lead to failure to admit membership in a high-risk group. The directed donor, despite the mandatory laboratory testing for human T-cell lymphotropic virus Type III and hepatitis B surface antigen, may in fact be less safe than the regular blood donor. Regular donors are allowed to donate only because past donations have not led to disease transmission — i.e., they are biologically tested. On the other hand, most directed donors are first-time donors.* The directed donor clearly forgoes the usual confidentiality. After all, the recipient knows whose blood he or she has received. This may place a donor in a position of legal jeopardy.

Widespread acceptance of the concept of directed donations seriously threatens the blood-transfusion system in this country. The current system of using altruistically motivated volunteer donors is designed to supply blood and blood products to patients, irrespective of the race, color, creed, or sex of the donor or recipient. A directed-donor system is basically a selfish system that fosters the hoarding of blood for some individuals to the exclusion of others. If such practice became widespread, it is possible that formerly altruistically motivated volunteer donors would begin to think that they should save themselves for situations in which friends or relatives might need their blood. Persons of wealth, power, and influence would be in a position of privilege when blood was concerned, whereas the elderly, poor, shy, or lonely in our society will be less likely to be able to influence friends or relatives to donate.

It might be justifiable to overlook these hazards of directed donation if we had good evidence that it was indeed safer than regularly donated blood. This has yet to be shown. The public has a right to expect the blood-banking community and the medical profession to remain objective and to distance themselves from the purely emotional aspects of this issue. In my view it would be highly irresponsible to place the national blood system in jeopardy by bowing to the often hysteria-based pressure for directed donations. Instead, we should increase our efforts to improve the safety of homologous blood and facilitate the use of autologous blood when feasible. To fail to promote this latter service would be as irresponsible as assenting to the clamor for directed donations.

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*Borchers G, Lord M. Impact of directed donation program on in-house blood collection: one hospital's experience. Transfusion 1985; 25:492. abstract

MORE ON PARTITIONING AND INACTIVATION OF AIDS VIRUS IN IMMUNE GLOBULIN PREPARATIONS

To the Editor: Prince et al. (Feb. 6 issue)[1] recently expressed concern that human T-cell lymphotropic virus Type III (HTLV-III) could be transmitted by intravenous immune globulin preparations. They suggested that conditions used to make intermediate Cohn–Oncley fractions did not inactivate spiked HTLV-III; however, they did not study partitioning of HTLV-III into the various fractions. We recently reported[2] the results of such studies.

HTLV-III was spiked into the various Cohn–Oncley fractions, and its partitioning between precipitates and supernatants, as well as inactivation, was studied by viral cultures and quantitative antigen measurements by antigen-capture enzyme-linked immunosorbent assay (ELISA). These studies showed that HTLV-III was partitioned and inactivated at multiple steps in the Cohn–Oncley process. At the most efficient step, HTLV-III was quantitatively (99.5 percent) partitioned into precipitate III, a fraction discarded during the manufacture of immune globulin. The cumulative effects of partitioning and inactivation resulted in a potential efficiency of virus removal of 10^{13} infectious units per milliliter. This efficiency is many orders of magnitude greater than would be needed to eliminate the small amounts of virus that potentially could be present in plasma pools used for immune globulin manufacture. Thus, the conjecture of Prince et al. that lyophilization of immune globulin would preserve HTLV-III infectivity is not relevant. In addition, not all intravenous preparations are lyophilized as they imply.

Surveillance studies of recipients of intravenous immune globulin, suggested by Prince et al., have been under way since the autumn of 1985. The number of patients is limited because most recipients are immunodeficient and would not be expected to acquire antibodies even if they were infected with HTLV-III. Furthermore, because immune globulin preparations may contain antibodies to HTLV-III,[3] patients receiving immune globulin on a long-term basis are unsuitable for study; antibody detected in serum samples from such patients could have been transferred passively.

Despite these limitations, 134 recipients (mostly patients with immune thrombocytopenia but a few with renal transplants or Kawasaki's disease) of intravenous immune globulin made by various manufacturers (data provided by J. Bussel, M.D., The New York Hospital–Cornell Medical Center, The Massachusetts Public Health Biologic Laboratories, Miles Laboratories, Travenol Laboratories, The Blood Transfusion Service of The Swiss Red Cross, Immuno AG, and Kabi Vitrum AB) have been followed for 2 to 24 months by means of ELISA and Western blotting of serum reactive by ELISA. Three patients had passively transferred antibodies; none produced HTLV-III antibodies.

Thus, the results of laboratory studies of the fractionation process and surveillance of immune globulin recipients corroborate epidemiologic observations indicating that receipt of immune globulin is not associated with the risk of the development of AIDS.

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DYSPLASTIC CARCINOID TUMOR AND AIDS-RELATED COMPLEX

To the Editor: The occurrence of small-cell tumors has been described in patients with AIDS.[1,2] Although these patients may have other malignant diseases (e.g., Kaposi's sarcoma or lymphoma),[2-4] small-cell tumors are rare. We report the development of a small-cell variant, dysplastic carcinoid tumor in a patient with the AIDS-related complex.

A 29-year-old white female prostitute who used intravenous drugs on a daily basis was admitted to our hospital with a five-month history of persistent malaise, anorexia, weight loss, and night sweats. Three weeks before admission a nonproductive cough developed and exercise tolerance decreased. Pertinent findings on physical examination included bilateral cervical, supraclavicular, axillary, and inguinal lymphadenopathy with at least one node larger than 1 cm in diameter in each area, the largest being 2.5 cm in diameter. Chest x-ray examination and CT scanning revealed a large anterior mediastinal mass. Specimens of two adjacent supraclavicular lymph nodes were obtained at biopsy, and pathological studies revealed prominent reactive follicular hyperplasia characteristic of the AIDS-related complex in one of the nodes,[5] whereas metastatic small-tumor cells effaced the normal lymphoid architecture in the other. Electron micrographs of the tumor showed it to be a dysplastic carcinoid. Viral, bacterial, and fungal cultures of the lymphoid tissue were negative. Further intensive workup, including bone marrow biopsy and culture, did not reveal other etiologic agents. The patient's serum was positive for HTLV-III antibodies on repeated enzyme-linked immunosorbent assays (Abbott), the results of which were confirmed by Western blot analysis (performed at the Rhode Island Blood Center).

The patient received three cycles of cyclophosphamide, vincristine, and etoposide in escalating doses, as well as radiation therapy to the primary lesion, which has been reduced in size by 30 percent. The peripheral adenopathy has also begun to regress, without the development of opportunistic infections.

Although she is at high risk for the development of AIDS, this patient is best categorized as having the AIDS-related complex. We cannot exclude the possibility that the enzyme-linked immunosorbent and Western blot assays were false positive. The patient has not had opportunistic infections or any of the malignant diseases associated with AIDS. Although she was a heavy smoker, the development of this type of tumor at this age is distinctly unusual. It is possible that an impairment of immune surveillance by antecedent retroviral infection facilitated the early appearance of this uncommon cancer.

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TOXIC KERATOPATHY ASSOCIATED WITH SURAMIN THERAPY

To the Editor: Suramin, an inhibitor of reverse transcriptase of HTLV-III,[1] has been used for many years in the treatment of African trypanosomiasis and onchocerciasis. It now is being used in clinical trials to evaluate its efficacy in patients with AIDS. We have observed a toxic keratopathy possibly due to suramin in these patients.

Five patients with AIDS who had no visual symptoms and who were treated with suramin at our institution underwent ophthalmologic evaluations including slit-lamp examinations. All received 500 mg to 1 g of intravenous suramin weekly after an initial dose of 200 mg. Two patients had been receiving suramin for approximately four months, with total doses of 17.2 and 15.2 g, respectively, and both had a diffuse subepithelial vortex keratopathy in both eyes, virtually identical to that seen in Fabry's disease and chloroquine keratopathy. A third patient received a total dose of 6.7 g over two months and had a vortex keratopathy inferiorly only, with a central haze in both eyes. A fourth patient who received suramin for seven weeks, for a total dose of 7.2 g, had a central corneal haze in both eyes that was due to fine punctate cream-colored deep epithelial or subepithelial opacities. The fifth patient received 3.2 g over five weeks and had only a few fine punctate cream-colored deep epithelial or subepithelial opacities centrally in both eyes. All patients had best corrected vision of 20/20 or better, and none had clinical evidence of a pigmentary retinopathy. No patient was taking any other drug known to cause a vortex keratopathy, including chloroquine, hydroxychloroquine, amiodarone, indomethacin, chlorpromazine, and clofazimine.[2,3] None had any physical findings suggestive of Fabry's disease, and the three patients tested for serum glycolipids had normal levels. The extent of keratopathy was related to the total dose of suramin. In contrast, 20 patients with AIDS or AIDS-related complex who had not received suramin had no evidence of keratopathy.

The idea that suramin could cause a vortex keratopathy is tenable, since it is a lysosomotropic compound that inhibits many lysosomal enzymes[4-6] and has induced systemic mucopolysaccharidosis when given to animals in large doses.[4,5] By analogy, the lysosomotropic drug chloroquine can induce a phenocopy of the vortex keratopathy observed in patients with Fabry's disease.[7] This phenocopy results from the inhibition by chloroquine of the activity of the

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Evidence Suggesting that Immune Globulin Preparations do not Transmit AIDS*

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INTRODUCTION

Transfusion of blood and blood products is recognized as a mode of transmission of the acquired immunodeficiency syndrome (AIDS) reported by the Centers for Disease Control (CDC) in the United States. Immune globulins have been implicated in none. This epidemiological finding gives reason to be encouraged about the safety of immune globulins, because millions of doses have been given since it was recognized that the lymphadenopathy virus human T-cell lymphotropic virus, Type III (LAV/HTLV-III) infected blood. Despite the absence of AIDS cases related to administration of immune globulins, their safety has been recently questioned.[1] Special concerns were raised about preparations for intravenous use, in contradistinction to intramuscular use, because direct 'blood-to-blood' contact might facilitate transmission of LAV/HTLV-III. Much of the controversy has been

* The opinions and assertions contained herein are the private views of the authors, and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

generated by several papers[1-4] reporting varying effectiveness in inactivating LAV/HTLV-III during fractionation of plasma to make immune globulin preparations.

In this chapter the limited *in vitro* and epidemiological data available concerning the safety of immune globulin are reviewed.

FATE OF LAV/HTLV-III DURING ETHANOL FRACTIONATION

Studies by Spire *et al.*[2] and Piszkiewicz *et al.*[3] both suggested that alcohol inactivated LAV/HTLV-III. In the former the effectiveness of several chemical agents in inactivating LAV/HTLV-III suspended in buffer was determined. Residual reverse transcriptase activity was very near zero following exposure to 19% ethanol for 10 minutes: the temperature of incubation was not stated, but presumably was ambient. In the report of Piszkiewicz *et al.*[3] LAV/HTLV-III was seeded into an aliquot of a cryoprecipitate-poor plasma pool made from 5000 donations from donors found previously not to have antibodies to LAV/HTLV-III. Samples were drawn at various times following addition of ethanol to a final concentration of 20% while maintaining the mixtures at −5°C and the median tissue culture infectious dose (ID_{50}) of each sample determined. The ID_{50} fell $10^{3.5}$ in 5 minutes, and the authors concluded that products made by cold ethanol fractionation carry a low risk of LAV/HTLV-III transmission.

In contrast to the findings of rapid inactivation cited above. Prince *et al.*[1] reported less optimistic results following seeding of LAV/HTLV-III into three supernatants (I, II + III, and III) made by the Cohn–Oncley process. After viral seeding the supernatants were kept between −2 and −5°C and cultured for LAV/HTLV-III after 2 and 24 hours. Under these conditions little or no inactivation occurred. Prince *et al.* did not explain the differences between their results and those of others.[2,3] Although they did not study partitioning, they noted that it might remove LAV/HTLV-III from immune globulins during fractionation.

We reported[4] that the Cohn–Oncley process either partitioned or inactivated sufficient LAV/HTLV-III that the risk of transmission of the virus by immune globulin seemed very unlikely. Briefly, portions of each fraction (including the starting plasma) leading to immune globulin were retained and seeded with sufficient LAV/HTLV-III to achieve a concentration in the fraction of between 10^4 and 10^6 *in vitro* infectious units per milliliter. After sampling to test for viral recovery, the next step in the process was carried out, yielding a precipitate and a supernatant that were both cultured in H9 cells for infectivity. Cultures were monitored by at least two of three methods: cytoplasmic antigen expression detected by immunofluorescence assay, and reverse transcriptase or antigen-capture enzyme-linked immunosorbent

assays (ELISA). The study design permitted evaluation of the relative effects of partitioning and inactivation at each step of the production process. The results were consistent with recovery from the supernatants plus the precipitates of more than 90% of the seeded virus.

The two most efficient partitioning steps were the initial cryoprecipitation, during which 95% of LAV/HTLV-III partitioned into the cryoprecipitate, which is not used in the manufacture of immune globulins, and the precipitation of fraction III. During this latter step, 99.5% of the seeded virus partitioned into the discarded precipitate III. In addition, during the precipitation of Fraction II from supernatant III with 25% ethanol for 24 hours at −5°C, the infectivity of both supernatant II and precipitate II was reduced from 10^4 to 10^1 or less *in vitro* infection units per milliliter. It was estimated that the cumulative reduction in *in vitro* infectious units by the entire production process was 10^{15}; several orders of magnitude more than would be likely to be present even in pools of several thousand donors unscreened for LAV/HTLV-III antibodies.

Most recently, Mitra *et al.*[5] confirmed the partitioning of the LAV strain of LAV/HTLV-III by cold ethanol fractionation. They could not detect virus in Fraction II, made from supernatant III containing 1.7×10^3 ID_{50} of virus. They also found a reduction of 10^5 infectious particles of mouse type C retrovirus if filtrate III at pH 4.0 (which contains 18% ethanol) was seeded and incubated at 22°C for 3 hours. In addition they described a reduction of 10^3 infectious particles if filtrate III at pH 4.0 (which also contains 18% ethanol) was seeded with LAV/HTLV-III and incubated at 5°C for 18 hours.

From the foregoing, it is clear that both partitioning and inactivation must be evaluated during *in vitro* studies from which conclusions are drawn about the potential infectivity of immune globulins manufactured by Cohn–Oncley fractionation. No single fractionation step or treatment is responsible for the reductions in infectivity observed; however, several combinations of ethanol treatment, partitioning, pH, and storage appear to reduce potential infectivity significantly, and perhaps quantitatively.

SURVEILLANCE OF RECIPIENTS OF INTRAVENOUS IMMUNE GLOBULIN

Several authors[6–8] have expressed concern about immune globulin safety based on the presence of antibodies to LAV/HTLV-III in commercial preparations. Passive transfer of these antibodies in hepatitis B immune globulin has been reported.[7] It is not suprising that immune globlin preparations contain antibodies to LAV/HTLV-III; the globulins are concentrated from large pools of plasma. Most lots available now and in the recent past were manufactured from plasma drawn before donors could be tested for anti-

bodies to LAV/HTLV-III. Data from our laboratory showing the appearance of these antibodies in immune globulin preparations are presented in Table 1. The emergence of lots containing antibodies to LAV/HTLV-III in the early 1980s lagged behind the emergence of clinical AIDS in the United States. Although the presence of antibodies to LAV/HTLV-III in individual units of donated blood is used as a criterion for removal from the blood supply, there are no data to suggest that lots of immune globulin containing these antibodies are unsafe. In could be argued, as it has been for the presence of antibodies to hepatitis B virus, that antibodies to LAV/HTLV-III in immune globulin may decrease the risk of transmitting the virus. There are no data to support this speculation. Without regard for its safety implications, the presence of LAV/HTLV-III antibodies in immune globulin preparations is relevant because it complicates surveillance studies due to the potential for passive antibody transfer.

Table 1 Detection of antibodies to LAV/HTLV-III in immune globulin preparations

Year of manufacture	Number tested	Negative (neat)	Reactive*	
			400*	> 1600†
1975–79	44	44	0	0
1980–81	3	3	0	0
1982	2	0	0	2
1983	5	0	1	4
1984	6	0	2	4
1985	13	1	4	8‡

* Reactive on ELISA, confirmed by Western blotting.
† Titer (reciprocal of dilution).
‡ One lot of hepatitis B immune globulin had a titer of greater than 256,000.

Manufacturers of intravenous immune globulin were asked by the United States Food and Drug Administration (FDA) to study recipients for seroconversion for antibodies to LAV/HTLV-III. The number of patients available for study was limited for two reasons. First, most patients who receive intravenous immune globulin are immunocompromised, and therefore the finding that they failed to develop antibodies to LAV/HTLV-III even if infected with the virus would not be instructive. Second, most patients who receive intravenous immune globulins are treated repeatedly and, as noted, antibody detected in their serum could have been passively transferred.

To date we have received data on 133 patients, most with idiopathic or immune thrombocytopenia, but a few with renal transplants or Kawasaki disease, who have been followed by ELISA between 6 and 24 months for the presence of LAV/HTLV-III antibodies. Passively transferred antibody

was detected by immunoblot in three patients; however, no patient produced antibody and therefore none was considered to have seroconverted.

Recently it was reported[9] that two patients who had received immune globulin intravenously for complex immune disorders later showed evidence of retroviremia. The authors concluded that iatrogenic transmission, i.e. by the immune globulin, could not be excluded. The clinical histories of the patients were extremely complicated, and no data substantiating the statement were provided. Other causes for the infections appeared equally or more likely.

SURVEILLANCE OF RECIPIENTS OF INTRAMUSCULAR IMMUNE GLOBULIN

CDC has been following a group of health-care workers who received immune globulin and/or hepatitis B immune globulin intramuscularly because they had been stuck by needles or other sharp instruments which were potentially contaminated by blood or other body fluids thought to be infectious for LAV/HTLV-III. To date, sera from 194 recipients have been assayed by ELISA for the presence of antibodies to LAV/HTLV-III. Antibody was present in the sera of two patients (G. McCray, personal communication, April 1986). In the first, no serum sample drawn prior to receiving the immune globulin was available, and the subject reported intimate contact with a member of a high-risk group. In the second, the immune globulin had been received 5 years prior to the stick exposure to LAV/HTLV-III and immune globulin was not given following exposure because the person had been vaccinated for hepatitis B in 1984. It is believed that her sero-coversion reflects LAV/HTLV-III infection as a result of the stick.

Although these findings support the safety of intramuscular immune globulin only, they provide additional confidence in the safety of all immune globulin preparations.

SUMMARY

Both laboratory studies of the effects of the Cohn-Oncley ethanol fractionation process on LAV/HTLV-III seeded into the various fractions and surveillance of recipients of immune globulin preparations corroborate epidemiologic observations indicating that receipt of immune globulin is not associated with a risk of developing AIDS or antibodies to LAV/HTLV-III.

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PARTITIONING AND INACTIVATION OF VIRUSES DURING ISOLATION OF ALBUMIN AND IMMUNOGLOBULINS BY COLD ETHANOL FRACTIONATION

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ABSTRACT

Albumin solutions invariably transmitted infectious hepatitis viruses before the introduction of pasteurisation in the final container. Immunoglobulin solutions (the older intramuscular as well as the current intravenous ones), on the other hand, only rarely transmitted hepatitis. The apparent safety of the latter was usually attributed to the presence of neutralizing antibodies and to the fractionation process. It was shown that viruses tend to concentrate in those fractions of the cold ethanol precipitation procedure which are used neither for albumin nor for immunoglobulin preparations. Additionally, ethanol alone inactivates some viruses, albeit much less at low temperatures than at room temperature. According to EC-directives, all manufacturers of stable blood products must introduce production steps which inactivate viruses or they have to prove that certain production steps, which are already being used, do inactivate viruses. In either case, the inactivation has to be validated with appropriate experiments. Procedures that are now recognized as virucidal are, e.g., pasteurisation (i.e., heating of the liquid product at 60°C for 10 hours), solvent/detergent (S/D) treatment, photodynamic treatment, or incubation at pH4 with pepsin.

Key words: Albumin, immunoglobulin, plasma fractionation, ethanol precipitation, virus inactivation.

INTRODUCTION

Transmission of viral diseases by protein solutions derived from human blood plasma has been recognized for over 40 years; however, not all products carried the same risk: albumin, plasma protein fraction, and immunoglobulins always belonged to the low-risk group, while coagulation factor concentrates (fibrinogen, factor VIII, factor IX) and other products (e.g., fibronectin, α_1-antitrypsin, C-1 inactivator) were high-risk preparations.

ALBUMIN

Albumin solutions (and plasma protein fraction) have not transmitted viral diseases since pasteurisation (i.e., heating at 60° for 10 hours in the presence of stabilizers) in the final container was introduced. However, inactivation of hepatitis B virus (HBV) is not as straightforward as it might appear: heating of an infectious plasma pool for only two or four hours was insufficient to inactivate HBV; albumin

prepared from this pool was apparently non-infectious when tested in small amounts (1 ml), but it transmitted HBV when infused in large amounts (100 ml). Heating completely abolished infectivity (reviewed in ref 1 with additional references). The rationale for introducing a heat step makes for historically interesting reading (2).

The viral antigen (HBsAg) partitions into different fractions during cold ethanol plasma fractionation: two groups of investigators found measurable, albeit small amounts of HBsAg in fraction V, but none in fraction II (3, 4). These laboratory-scale experiments helped to explain the infectivity of unpasteurised albumin solutions prepared from large pools.

Although little evidence is available to prove the point, it is likely that a small proportion of the human immunodeficiency virus (HIV) and the hepatitis C virus (HCV) – which were certainly present in pools before introduction of the respective screening tests, and most likely also after screening, although in much reduced amounts – is present in fraction V. Obviously, both viruses are inactivated by the pasteurisation step, since no transmission of either virus has been reported with albumin. The faith in the effectiveness of pasteurisation was so general that we had to wait until 1987 for laboratory proof that HIV loses its infectivity when pasteurised in the presence of albumin solutions (5). This experimental evidence was not superfluous, since it is well known that stabilizers (and other solutes) may prevent inactivation of viruses as well as of proteins.

IMMUNOGLOBULINS

Alleged transmissions of viruses by immunoglobulin preparations which have been reported in the literature are listed in Table I. A complete and accurate survey of all incidents is difficult, one reason being that the same episode is often reported more than once in different journals. The anti-D implicated in the incident reported elsewhere (6) was prepared by unknown methods from the plasma of donors vaccinated with Rh_0(D) positive erythrocytes obtained from a carrier of non-A, non-B hepatitis. Plasma donors as well as the recipients of the anti-D and many of their infants were infected (1). The preparation referred to by Lane (7) was produced by Kistler/Nitschmann cold ethanol fractionation (8); ethanol was subsequently removed by gel filtration in the presence of maltose (9). Production of this preparation was discontinued after this incident, which occurred in the trial phase. Ochs et al. (10) used a preparation which was isolated by Cohn/Oncley fractionation and treated with polyethylene glycol and DEAE-Sephadex. The two lots had been prepared from the same batch of fraction II in a pilot plant; additional batches manufactured in a production facility by the same method did not cause any problems in 600 patients, 23 of whom had primary immunodeficiency (11). The episodes observed by several authors (12, 13, 14) are probably due to failures of Good Manufacturing Practice. The two cases reported by Webster et al. (15) were clinically very complicated and implication of the immunoglublin preparation in the transmission of the virus could never be established with any degree of certainty.

As shown in Table I, the main concern of the clinicians is potential transmission of non-A, non-B hepatitis by immunoglobulin preparations. However, as repeated in a recent editorial, «No intravenous immunoglobulin preparation licensed in the

Table I: Alleged transmissions of viruses (excluding HBV) by immunoglobulin preparations.

Authors	Virus	Number of patients	Method of preparation
Renger et al. (6)	HCV	106/106	(anti-D)
Lane et al. (7)	HCV	12/12	K/N + Sephadex G 25
Ochs et al. (10)	HCV	7/16	C/O + PEG + DEAE-Sephadex
Weiland et al. (12)	HCV	4 (case study)	?
Webster et al. (15)	HIV	2 (case study)	K/N + pH4/pepsin
Björkander et al. (13)	HCV	16/77	C/O + DEAE-Sephadex
Williams et al. (14)	HCV	4/34	K/N + pH4/pepsin

K/N: Kistler/Nitschmann cold ethanol fractionation.

C/O: Cohn/Oncley cold ethanol fractionation.

United States and produced in manufacturing (as opposed to pilot) facilities has been associated with the transmission of non-A, non-B hepatitis.» (16).

As mentioned above, no HBsAg was found in fraction II (3, 4); on the other hand, a substantial part of the anti-HBs present in the starting plasma was recovered – not surprisingly – in fraction II (17). Similar experiments were carried out by the FDA in 1986 to trace the fate of HIV during Cohn/Oncley plasma fractionation (18). Their results were confirmed and extended to other fractionation schemes by other groups (19, 20). Taken together, these experiments demonstrated that HIV preferentially partitioned into the precipitates at every separation step. Treatment with cold ethanol alone only had a minor inactivating effect on HIV. Freezing and thawing of the pastes (which is routinely done in production for logistic reasons) also inactivated about 1 $\log_{10}$ of HIV.

The presence of antibodies against HIV in immunoglobulin preparations made from unscreened donations has been known at least since 1985 (21). Even though this early publication already warns against equating the presence of anti-HIV antibody in a final product with infectivity, the literature abounds, to this day, with examples of authors expressing exactly this concern. The tests used to detect anti-HIV antibody were developed for use on individual plasma donations, not on products made from pools of thousands of donations. We have shown that Western blot kits from three different manufacturers gave useless results with 47 batches of intravenous immunoglobulin preparations: the patterns never looked like those obtained with positive individual plasmas, different kits gave different patterns with the same batch, and the tests did not allow products made from plasma that included or excluded anti-HIV positive donations to be distinguished (22). These experiments were done with the «natural» antigens that were available at the time; we would expect, however, similar spurious results if the study could be repeated with today's recombinant antigens.

Not surprisingly, anti-HCV antibody was found in practically all immunoglobulin preparations that were recently tested with three different assays (23). The conclusion the authors draw that «Contraction of NANB hepatitis by patients infused with anti-HCV-positive IVIG indirectly indicates the presence of transmissible virus» is not supported by evidence. Selection of the donors has a clear effect on

viral markers: while only 0.3% of donations collected from voluntary donations were confirmed positive for anti-HCV antibody, this figure was 10.3% for commercial donations (24). Apart from such differences, which can be documented, unpaid donations probably also carry lower risks for other potential infections which cannot be detected by screening (25).

Since it is not (yet) possible to grow and titrate HCV in the laboratory, its behaviour during fractionation and susceptibility to inactivation have to be inferred indirectly. The FDA has taken a very interesting approach: they fractionated, according to Cohn/Oncley, a plasma pool prepared from donations that were all anti-HCV positive and followed, with polymerase chain reactions (PCR), the fate of HCV RNA in the various fractions. By diluting the samples before PCR it was possible to titrate the samples and to express their activity in «PCR units», which are roughly equivalent to a chimpanzee infectious dose. As with other viruses, HCV RNA was found predominantly in the precipitates (cryoprecipitate, fraction I, fraction III); trace amounts were also detected in fraction II. Overall reduction in HCV RNA from plasma to fraction II amounted to 4.7×10^4. Low levels of HCV RNA were also found in supernatant II+III, from which albumin is prepared; obviously, pasteurisation destroys whatever infectivity may be left in these fractions (26). As the authors point out, the presence of HCV RNA in fraction II should not be equated with infectivity, since other production steps may inactivate viruses, the entity they detected may be naked RNA, and antibodies may neutralize free virus. If the virus components are dissociated by the fractionation conditions, HCV RNA and infectivity may not co-distribute.

We used a somewhat different approach to study our own fractionation method (8). We first showed that Semliki Forest Virus (SFV; a togavirus which is related to HCV) was essentially insensitive to 10, 20, 40, and 80% ethanol at 0°C, while it was rapidly inactivated by 40 and 80% ethanol at 20°C; there was a slow decline in activity in the presence of 20% ethanol at 20°C. We then obtained samples which were withdrawn by the production department immediately before precipitation, spiked them with SFV and carried out the precipitation. The virus was titrated both in the supernatant and in the precipitate. Parallel experiments were done with SFV radiolabelled either with ^{35}S-methionine or with ^{3}H-uridine. We usually found about 90% of both ^{35}S- and ^{3}H-radioactivity (loosely or non-incorporated radioactivity accounts for about 5% of the total in both preparations), and 80% or more of the titrated activity in the precipitates. The precipitate GG, from which immunoglobulins are prepared (8), still contains SFV; reduction of virus load from starting plasma to precipitate GG was about 3 $\log_{10}$. Our results confirm that there is a considerable partitioning effect, which lowers the burden of HCV-like viruses in the plasma fraction from which immunoglobulins are prepared. Other production steps, such as freezing and thawing of pastes, which are known to contribute to virus inactivation, were not considered in these experiments.

There are other steps in the manufacturing process of immunoglobulins which contribute to virus inactivation; the following are two examples of such steps which have recently been validated. One manufacturer showed that pasteurisation of various formulations inactivated marker viruses (bovine viral diarrhoea virus (BVDV); tick-borne encephalitis virus (TBEV); yellow fever virus (YFV); HIV-1/2, herpes simplex virus type 1 (HSV-1); polio virus type 1) to below detection limit. Inactivation of YFV took about 6 hours, while HIV-2 was already completely

inactivated within 0.5 hours. The normally used pasteurisation time of 10 hours therefore guarantees in most cases a comfortable margin of safety (27).

It was shown in 1988 that pepsin treatment at pH4 inactivated vaccinia virus, SFV, HSV and mumps virus, but not poliovirus (28). Our own experiments, which were carried out under production conditions, also showed inactivation of HIV, HSV, human cytomegalovirus (HCMV), vesicular stomatitis virus (VSV) and SFV. Except in the case of VSV pH4 alone was sufficient to inactivate all viruses tested below detection limit; VSV was completely inactivated at pH4 in the presence of pepsin (29).

The final safety test for an immunoglobulin preparation remains its clinical performance in retrospective and prospective trials. We showed that our pH4/pepsin-treated intravenous immunoglobulin preparation did not transmit non-A, non-B hepatitis or any other viral disease to 68 patients with primary immunodeficiency disorders who were enrolled in a prospective, multicentre trial (30). Furthermore, anecdotal evidence indicates that no transmission of either HIV or NANBHV occurred with this preparation, not even when it was prepared from plasma which had not been tested for anti-HIV or anti-HCV; > $3x10^7$ g have been used worldwide so far.

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VIRAL SAFETY OF SOLVENT-DETERGENT TREATED BLOOD PRODUCTS

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ABSTRACT

Laboratory research commencing in 1982 led to licensing in the United States in 1985 of a solvent/detergent (SD)-treated anti-haemophilic factor (AHF) concentrate. Licensing was based on (a) studies demonstrating the inactivation of several marker viruses [vesicular stomatitis virus (VSV), Sindbis virus, Sendai virus], human immunodeficiency virus (HIV), hepatitis B virus (HBV), and non-A, non-B hepatitis virus [NANBHV; now known to be principally hepatitis C virus (HCV)] added to AHF just before treatment, (b) the realization that the principal viruses of concern in a transfusion setting (e.g. HIV, HBV, NANBHV) were all lipid-enveloped, and (c) laboratory, preclinical and clinical evidence indicating that AHF and other proteins present in the preparation were unaffected. The applicability of the SD method to a wide range of products and preparations, high process recoveries, and a growing body of viral safety information linked with the failure of several other virus inactivation methods to eliminate hepatitis transmission fostered the adoption of SD technology by more than 50 organizations world-wide. SD mixtures are now used in the preparation of products as diverse as intermediate purity and monoclonal antibody purified AHF and other coagulation factor concentrates, fibrin glue, normal and hyperimmune IgG and IgM preparations including those derived from tissue culture, plasma for transfusion, and various diagnostic controls. Over four million doses of SD-treated products have been administered, and numerous laboratory and clinical studies designed to assess virus safety have been conducted. SD treatment has been shown to inactivate $\geq 10^{5.3}$ tissue culture infectious doses ($TCID_{50}$) of VSV, $\geq 10^{6.8}$ $TCID_{50}$ of Sindbis virus, $\geq 10^{6.0}$ $TCID_{50}$ of Sendai virus, $\geq 10^{7.1}$ duck infectious doses of duck HBV, $\geq 10^{7.0}$ $TCID_{50}$ of HIV-1, $\geq 10^{6.0}$ $TCID_{50}$ of HIV-2, $\geq 10^{6.4}$ chimpanzee infectious doses (CID_{50}) of HBV, $\geq 10^{5.0}$ CID_{50} of HCV, $\geq 10^{6.0}$ $TCID_{50}$ of cytomegalovirus, $\geq 10^{6.3}$ $TCID_{50}$ of herpes simplex virus type 1, $\geq 10^{6.0}$ $TCID_{50}$ of PI-1, $\geq 10^{6.0}$ $TCID_{50}$ of murine leukemia virus (Mov-3), $\geq 10^{6.0}$ $TCID_{50}$ of murine xenotropic virus, and $\geq 10^{5.0}$ $TCID_{50}$ of Rauscher murine leukemia ecotropic virus. Moreover, in ten prospective clinical studies, 0/53, 0/427, and 0/455 patients susceptible to HBV, NANBHV (HCV), and HIV became infected on follow-up. These results contrast with the high rate of virus transmission observed previously in haemophiliacs and suggest that coagulation factor concentrates and other SD-treated products prepared from plasma pools are now safer than the individual units from which they were derived.

INTRODUCTION

The solvent/detergent (SD) virus inactivation method, licensed by the U.S. F.D.A. in 1985 for use in the manufacture of an AHF concentrate, has gained widespread popularity in the manufacture of biologics. This popularity arises from the high virucidal action of SD treatment, high protein compatibility, ease of insertion into virtually any pre-existing or newly developed purification process, and the availability of extensive documentation, speeding both implementation and

regulatory review. This review attempts to provide a historical context and an up-to-date summary of SD usage.

Basis for Regulatory Approval in 1985

The use of SD to eliminate virus infectivity of blood protein fractions was a result of the tradition of using ethyl ether and Tween 80 to distinguish lipid enveloped from protein-coated viruses. In 1982, the causative agent of non-A, non-B hepatitis (NANBH) had not been isolated, though infectious serum samples were available for study. Based on Tween/ether sensitivity of the Hutchinson isolate, we were among the first to demonstrate that at least this strain of NANBHV is lipid-enveloped (1). Simultaneously, we demonstrated that hepatitis B virus is also inactivated by this method. A similar conclusion regarding NANBHV was reached using chloroform (2). Exploration of the effect of ether/Tween treatment on selected plasma proteins indicated that their biological function was largely retained.

Because ethyl ether is explosive and its use is not practical in most manufacturing environments, these seminal findings fostered a wider examination of solvent/detergent pairs, ultimately leading to the substitution of ether with tri(n-butyl)phosphate (TNBP). The use of TNBP was favoured because it is non-volatile and non-flammable, it was used previously in an inactivating step in the manufacture of viral vaccines (3), it could be removed virtually completely from treated solutions, achieving levels which were four to five orders of magnitude lower than the toxicological threshold, virucidal potency was higher than that observed with ether (based on the rate of virus killing), and the retention of protein functional activity was complete or nearly complete. We initially substituted sodium cholate for Tween 80 because cholate is a naturally occurring detergent with an extremely large margin of safety on infusion, and because cholate, but not Tween 80, could be readily removed during the AHF purification procedure we then used. Nonetheless, because the rate of virus killing and protein compatibility are both higher with non-ionic detergents such as Tween 80 or Triton X-100 than with cholate, we and others have turned to their use.

Confidence that use of SD treatment would enhance the safety of coagulation factor concentrates came from several virus kill studies, each important in its own right. First, vesicular stomatitis virus (VSV), Sindbis virus, and Sendai virus, used as markers, were all killed relatively rapidly, and to the extent of challenge ($\geq 10^7$ tissue culture infectious doses or $TCID_{50}$), on exposure of our AHF concentrate to 0.3% TNBP and 0.2% sodium cholate at 30°C for six hours. The non-enveloped encephalomyocarditis virus, used as a control, was not inactivated (4). Second, based on the absence of transmission of hepatitis to chimpanzees on intravenous infusion of a pool of AHF concentrates, derived from 13 different lots and five U.S. manufacturers and treated by us with TNBP/cholate, we concluded that it was highly likely that all forms of blood-borne NANBHV were lipid enveloped (5). It should be noted that the AHF concentrates which we used were not derived from donors screened by any of the screening test methods introduced in 1985 or later, and were neither virally inactivated nor highly purified. Indeed, subsequent challenge of the chimpanzees with the untreated pool resulted in classical NANBH. Third, we showed that $\geq 10^5$ chimpanzee infectious doses (CID_{50}) of each of HBV and HCV were inactivated on treatment of an AHF concentrate with TNBP/

cholate (5). Fourth, shortly following isolation and growth of HIV in long-term culture by Dr. R. Gallo and his colleagues (6), we showed that HIV was rapidly and completely inactivated to the extent of challenge on treatment of AHF with TNBP/cholate (7).

Importantly, process recovery of factor VIII procoagulant activity was 80%, the reagents were removed easily by gel filtration, neo-immunogen formation did not occur as judged by the absence of detectable antibodies in rabbit sera raised against SD-AHF, which did not react with untreated AHI and, finally, treated and untreated AHF was demonstrated to have the same circulatory recovery and half-life on administration to haemophiliacs, in a controlled, cross-over trial.

Based on the above findings, and the heightened concern over viruses, especially HIV, in coagulation factor concentrates, AHF treated with 0.3% TNBP and 0.2% sodium cholate at 30°C for at least six hours was licensed by the U.S. F.D.A. in 1985.

Additional Evidence of Protein Compatibility

Shortly after licensing of SD-AHF in 1985, we studied the effect of SD treatment on a large number of blood protein preparations, measuring virus kill and protein functional activity. In each case, both virus killing and retention of protein functional activity were high (8). A wide variety of proteins of differing structures and functions was examined, including factor VIII, factor IX, fibrinogen, fibronectin, specific IgG antibodies, haemoglobin, alpha-interferon, and tumour necrosis factor. High protein concentration or the presence of even 2M glycine did not affect the outcome. A high lipid content, such as is found in whole plasma, could be accommodated by raising the concentration of TNBP, raising the temperature of treatment, or both. Based on this study, we concluded that SD treatment was broadly applicable. This has since been confirmed in published and unpublished clinical studies assessing circulatory recovery and/or half-life of protein preparations as diverse as intermediate purity (9) and monoclonal antibody-purified factor VIII (10, 11), factor IX (12, 13), fibrinogen, factor V, normal (14) and hyperimmune IgG24 monoclonal IgM, etc.

Additional Pre-Clinical Evidence of Virus Safety

Since our initial investigation, the number and titre of viruses used in validation studies have increased substantially (Table I). In each case, regardless of the enveloped virus used or challenge dose, virus kill was complete. Additionally, we have made extensive use of the chimpanzee model to verify that HBV, HCV, and hepatitis delta virus (HDV) were inactivated regardless of the TNBP/detergent pair or the protein solution used (Table II). Kill was complete in each case and, since the treated solutions were infused without benefit of other steps in the purification process, and without use of reagent removal methods which might be expected to decrease the challenge dose of virus, the total observed killing can be ascribed to the SD treatment per se.

Given that chimpanzees are an endangered species and that the cost of studies in chimpanzees is high, one cannot realistically determine the rate of kill of HBV

Table 1. Virus inactivation with TNBP/detergent.

Virus	Inactivation ($\log_{10}$)
VSV	≥ 9.2
Sindbis	≥ 8.8
Sendai	≥ 6.0
HBV	≥ 6.0
HCV	≥ 5.0
HIV-1	≥ 11.0
HIV-2	≥ 6.0
DHBV	≥ 7.3
CMV	≥ 6.0
HSV-1	≥ 5.8
VEE	≥ 6.0
PI-1	≥ 4.0
MuLV (Mov - 3)	≥ 6.0
Murine xenotropic virus	≥ 4.0*
Rauscher murine leukemia ecotropic	≥ 2.0*

* Virus was reduced to below level of detection within minutes; at 5.5 hours virus was respiked into solution and completely inactivated.

or HCV. Since duck HBV (DHBV) has been proposed as a surrogate for human HBV, in collaboration with Dr. Alex Sito of Quality Biotech, we determined the rate and extent of kill of this virus on SD treatment of plasma. Because our intent, in part, was to validate the model, we also determined the rate and extent of kill of DHBV when added to AHF and pasteurized at 60°C in the presence of 50% sucrose and 2.2 M glycine, stabilization conditions in common use today. On treatment of plasma with 1 % TNBP and 1% Triton X- 100 at 30°C, 10^8 ID_{50} of DHBV were inactivated in 90 minutes, and complete inactivation of the challenge inoculum (≥10^{11} ID_{50}) occurred at a time between the 1.5 and 4 hour sampling points. By comparison, only 10^7 ID_{50} of DHBV were inactivated with 10 hours of pasteurization (Fig. 1). These results generally conform to the expected, since SD has been demonstrated to kill ≥ 10^8 chimp infectious doses (CID_{50}) of HBV while pasteurization at 60°C in one case was reported to inactivate approximately 10^4 CID_{50} of HBV (15), and in another case, the combination of removal and inactivation was shown to eliminate ≥ 10^{10} CID_{50} of HBV (16). Moreover, these results are also in accord with the considerable stabilization afforded to other viruses when pasteurized at 60°C in the presence of stabilizers (17).

Evidence of Virus Safety in Formal Clinical Trials

The first trial, which used our own TNBP/cholate-treated AHF, was conducted jointly with the U.S. F.D.A. (18). Of the 17 patients entered and followed for

Table II: Summary of chimpanzee studies.

Test material	Treatment conditions	Virus dose	Number of animals	Results - positive/total
Pool of commercial AHF concentrate	1	endogenous*	2	0/2
HCV added to an AHF concentrate	**2**	**10^4**	**2**	**0/2**
HBV added to an AHF concentrate	2	10^4	2	0/2
HDV added to an AHF concentrate	**2**	**10^4**	**2**	**0/2**
HCV added to Cohn fraction II	3	10^5	2	0/2
HCV added to plasma	**4**	**10^5**	**2**	**0/2**
HBV added to plasma	4	10^5	2	0/2
HBV added to plasma	**5**	**10^5**	**2**	**0/2****
HCV added to plasma	5	10^5	2	0/2

1. 0.3% TNBP, 0.2% sodium cholate at 24°C for six hours.
2. 0.3% TNBP, 0.2% sodium cholate at 30°C for six hours.
3. 0.3% TNBP, 1% Tween 80 at 24°C for six hours.
4. 2% TNBP at 37°C for four hours.
5. 1% TNBP, 1% Triton X-100 at 30°C for four hours.

* Pool of 13 lots from five U.S. manufacturers.

** Animal 326 died of anaesthesia-related incident after 28 weeks of follow-up.

hepatitis transmission for at least six months, 13 had not previously received blood products and three had only received three or fewer units of a single donor product. All had been vaccinated against HBV. ALT measurements were made at two-week intervals for the first eight weeks and then monthly for at least six months. Since none of the ALT measurements exceeded (or even approached) twice the upper limit of normal, we concluded that 0/17 patients developed signs of NANBH. Samples from 12 patients were also tested for anti-HCV, once this test became available; all were negative (19). Additionally, 0/18 patients developed anti-HIV antibody. It should be emphasized that much of this study was performed before the availability of HCV-antibody testing and before routine implementation of testing for HIV-antibody, ALT, or hepatitis B core antibody. Additionally, this concentrate did not have the advantage of chromatographic purification. Since use of these tests should decrease viral loads by 10- to 100-fold, and ion exchange and monoclonal antibody affinity chromatography have been shown to reduce viral loads (20, 21), modern concentrates which employ SD treatment should have an even higher probability of safety.

Since this first study, numerous other investigations have been carried out on SD treated products (Table III). In aggregate, 10.7 million units were infused, and 0/53, 0/427, and 0/455 of the patients developed signs of HBV, HCV, and HIV transmission, respectively. It is interesting to note that one centre prepared an SD-AHF (intermediate purity) and a prothrombin-complex concentrate (PCC) which

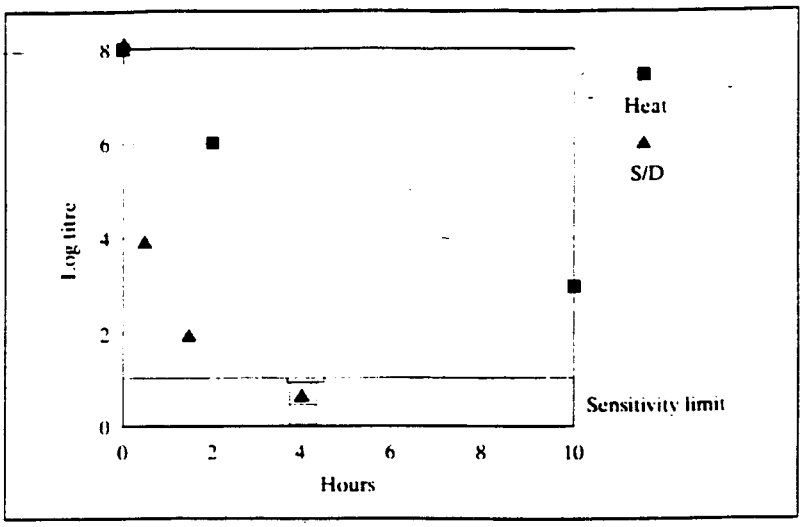

Fig. 1: Inactivation of duck HBV. Solvent/detergent vs pasteurization.

was heated in the lyophilized state at 60°C for 72 hours from the same plasma pool. The SD-AHF proved to be safe; the heated PCC transmitted HBV and HCV (22). This finding is all the more remarkable since these viruses are known to concentrate away from the PCC fraction and into the AHF fraction during cryoprecipitation; the AHF was subjected to little additional purification, and the PCC was chromatographically purified.

Routine Clinical Use

SD-treated products have been approved for routine use in numerous countries, including Argentina, Australia, Austria, Belgium, Canada, Czechoslovakia, Denmark, Finland, France, Germany, Israel, Italy, Japan, Korea, the Netherlands, Norway, Poland, Portugal, Saudi Arabia, South Africa, Spain, Sweden, Switzerland, the United Kingdom, the United States and Venezuela. Products which are approved and the approximate number of doses transfused following approval are given in Table IV. It should be noted that the 3.8 million doses of AHF transfused represents over 45,000 man-years of treatment, assuming an average infusion of 80,000 IU per man-year. Based on current usage patterns, approximately two thirds of the AHF transfused in North America, western Europe, and Japan is SD-treated. Throughout this time period, not a single case of HBV, HCV, or HIV transmission has been reported. To place this in perspective, before 1985, based on studies in chimpanzees and on clinical studies of the first of the dry heat-treated AHF concentrates available in the U.S., essentially every vial contained HCV, and transmission of HIV and HBV occurred frequently.

Table III: Viral safety of solvent/detergent-treated coagulation factors.

References	Concentrate	Total units infused (approx.)	Positive/Total		
			HBV	NANBHV	HIV
Horowitz MS et al, 1988 Horowitz MS et al, 1990	AHF	145,000	na	0/17	0/18
Guerois C et al, 1991	AHF FIX AHF, FIX	ua ua	na na	0/27 0/5 0/4	0/18 0/5
Brackman HH, 1992	AHF	ua	na	0/165	0/49
Gonzaga AL, 1991	PCC AHF	1,104,600 5,476,000	0/16 0/16	0/21 0/22	0/21 0/124
Panicucci F et al, 1990	AHF	1,371,600	na	0/23	0/40
Di Paolantonio T et al, 1992	AHF	1,272,000	na	na	0/29
Mariani G, 1992	AHF	165,000	0/14	0/31	0/31
Gomperts E, 1991	AHF	ua	na	0/109	0/60
Perret BA et al, 1989	AHF PCC	541,000 265,000	na na	na na	0/18 0/8
Perret BA et al, 1991	AHF	158,600	na	na	0/6
Peerlinck K et al, 1991	AHF	ua	0/3	0/7	0/19
		10,498,800	0/53	0/427	0/455

Table IV: Reported usage of SD-treated products 1985-October, 1992.

Product	Units	Doses (approximate)
FVII	1,9 MU*	1,900
FVIIa	2.6 MU	2,600
FVIII	3,864 MU	3,864,000
FIX	187 MU	187,000
PPSB	69 MU	69,000
CPPA	10 MU	4,400
Fibrin glue	75,931 mL	15,190
Fibrinogen	75,688 g	19,000
Imig & Ivig	318,000 g	63,600
Mab IgM	2,697 units	2,697
Plasma	7,707 units	1,926
		4,231,313

* million units

Based on estimates of the viral load present in plasma pools and removal and inactivation during processing, we conclude that vials of modern plasma-derived coagulation factor concentrates prepared with the SD method have less than one chance in 10^{-6}, 10^{-7}, and 10^{-9} of having HIV, HBV, or HCV present, respectively (23).

HIVIG

In response to the AIDS epidemic, we developed procedures for the manufacture of a hyperimmune anti-HIV immune globulin derived from individuals infected with HIV but without overt signs of disease. Because of the potential danger to fractionation technicians, the plasma was virally inactivated with TNBP at the time of pooling. Subsequently, the IgG was isolated by Cohn-Oncley cold ethanol fractionation, and was given a second treatment with SD to assure further safety to the recipient. As a safety test, both the virally inactivated plasma and the purified final product were injected into chimpanzees. Despite being prepared exclusively from high risk donors, neither sample resulted in the transmission of HIV or of hepatitis (24). Moreover, the injected IgG exhibited a normal circulatory recovery and half-life. In a subsequent study, the prevention of HIV infection on challenge of an injected animal with 10 CID_{50} of HIV (25) (but not 200 CID_{50} (24)) has fostered the evaluation of HIVIG, prepared using SD treatment, in the United States and Europe.

Introduction Into Manufacture

Given the reproducible and predictable viral killing achieved with numerous different protein preparations, the SD method can figuratively be considered as a cassette which can be inserted into virtually any purification scheme. While the method was developed for use with blood protein solutions, SD treatments have been applied successfully to animal sera for use in tissue culture, both IgG and IgM monoclonal antibodies, products of recombinant DNA technology (especially when cell-culture derived), and to diagnostic reagents (26). To provide assurance that virus kill occurs as expected, NYBC requires that the rate and extent of kill of VSV and Sindbis virus be measured in each preparation and the results compared with the kill achieved on treatment of the NYBC AHF concentrate. If the rate of kill is comparable, it is our belief that the safety exhibited by our AHF concentrate and by other SD-treated AHF concentrates purified by a similar method would be exhibited by the new preparation. Other factors are also important, e.g., whether the viral load in plasma used in the manufacture of the new preparation is substantially higher than previously encountered. Since our preparation was of low purity, and since cryoprecipitation serves to concentrate virus, most new preparations will have a lower viral load than was present in our original AHF concentrate.

SD-Plasma

At one time, the viral danger presented by coagulation factor concentrates greatly exceeded the danger from single donor products, e.g., fresh frozen plasma (FFP) or cryoprecipitate. With the development of powerful virus inactivation

procedures. AHF concentrates and other virally inactivated plasma products are now safer than the individual donations from which they were derived. This safety has encouraged the development of SD-Plasma as a substitute for FFP (27). Briefly, units of FFP are combined, thawed, treated with 1% TNBP and 1% Triton X-100 at 30°C for four hours, the reagents removed by hydrophobic chromatography, and the final product sterile-filtered, frozen and, optionally, lyophilized. Virus inactivation has been extensively validated. Under these conditions of SD treatment, the rate of VSV and Sindbis virus killing exceeds that observed with AHF concentrates, treated either with TNBP/cholate or TNBP/Tween. We have also shown that $\geq 10^{6}$ CID_{50} of HBV, $\geq 10^{5}$ CID_{50} of HCV, and $\geq 10^{7.2}$ $TCID_{50}$ of HIV are killed. Additionally, because of our interest in validating the duck HBV model (described above), we have shown that $\geq 10^{7.3}$ ID_{50} of DHBV are inactivated. Coagulation factor content is high and in the range expected for FFP, and clinical results are excellent (28, 29, 30). SD-Plasma is now in routine use in France and Germany, and a Product Licence Application was submitted to the U.S. F.D.A. in February, 1993.

Non-Enveloped Viruses

The SD method does not inactivate non-enveloped viruses, and the foundation for use in a blood transfusion setting was the realization that the principal viruses of concern (HBV, all forms of NANBHV including HCV, all retroviruses including HIV) were all enveloped. Nonetheless, two viruses, parvovirus and, more rarely, hepatitis A virus (HAV), have been transmitted by cell-free blood products and should be discussed.

Parvovirus is a ubiquitous virus causing fifth disease, so-named because it was the fifth recognized exanthema of childhood (31, 32). Infections are frequently asymptomatic, and because antibody is neutralizing, self-limiting. A decline in erythroid progenitor cells, seen in some individuals, would only appear to be serious in patients with another underlying haematologic disorder, for example, thalassemia. Some reports indicate that the foetus may be at risk for the development of hydrops foetalis if the mother is infected with B19 during pregnancy, though in most cases, the foetus was reported not to be affected. Since parvovirus is also heat-stable, current evidence indicates that essentially all coagulation factor concentrates can transmit parvovirus (33, 34), although a reduced rate of transmission was claimed for a preparation heated at 80°C for 72 hours (34). Most blood recipients have already been exposed and have immunity, including 50-60% of the general population and virtually all adult hemophiliacs.

A more extensive discussion of HAV would appear to be in order since there have been several recent reports of hepatitis A amongst recipients of an ion exchange chromatography purified, SD-treated, AHF concentrate (35, 36, 37). Hepatitis A virus is a picornaviruses. It is generally transmitted by the faecal-oral route and is highly prevalent in the developing world where ≥50% of the population may be infected in early childhood. In countries with advanced standards of hygiene, infections may occur at any time throughout life since most adults are not immune. In infancy, most infections are subclinical, while about 25-50% of infected adults develop overt hepatitis. The incidence of HAV infection in developed countries is highly variable depending on the occurrence of epidemics, but it has

been rather stable overall in the United States in recent decades. The infection is in all cases self-limited, with viraemia persisting for only a few days or weeks. Except in the rare case of fulminant infection, exposure is followed by complete recovery without chronic residua such as cirrhosis.

HAV transmission to blood recipients has occurred only rarely. For example, HAV transmission did not occur in any of the numerous, prospective studies of single donor products designed to monitor hepatitis transmission and conducted in the past 10-15 years (Table V). Moreover, HAV was not transmitted to chimpanzees in any of the evaluations assessing coagulation factor concentrates, including the challenge phase when animals received the non-virally inactivated control. Moreover, we are aware of only one case of HAV reported in any of the numerous, prospective safety trials conducted with any coagulation factor concentrate, independent of viral inactivation method used (38).

Regarding the recent HAV outbreaks, the first was associated with an SD-treated AHF concentrate manufactured at one site in Italy from U.S. commercial plasma. At least 41 cases of HA, widely dispersed throughout Italy, were described (35). Thirty-eight (93%) involved icteric disease. Lot tracking indicated that if SD-AHF was the vector in all cases, at least 12 different lots of product were involved. The AHF production method used ion-exchange chromatography in addition to SD treatment. Between batches, the column was regenerated and sanitized with NaOH. Interviews with and serological screening of fractionation employees ruled

Table V: Absence of hepatitis A transmission on transfusion of blood.

Reference	Country	Number of patients	Units transfused	Number					
				PTH	HA	NANBH	HB	CMV	EBV
Aach et al. 1981	United States	1,528	5,564	171	0	156	15		
Alter et al. 1981	United States	283	3,359	36	0	35			
Katchaki et al. 1981	Netherlands	380	740	15	0	13	0	1	1
Cossart et al. 1982	Australia	842	4,789	18	0	14	3	1	
Grillner et al. 1982	Sweden	74	814	15	0	14	0	1	
Collins et al. 1983	Great Britain	248	1,796	38	0	38	0	0	
Hernandez et al. 1983	Spain	230	936	40	0	29	10	1	
Tremulada et al. 1983	Italy	246	1,500	34	0	29	+	+	
Tur-Kaspa et al. 1983	Israel	50	606	4	0	4	0		
Aymard et al. 1986	France	64	447	5	0	4	0	1	
Colombo et al. 1987	Italy	676	4,813	96	0	92	3		
Sugg et al. 1988	Germany	417	2,270	16	0	15	1		
Widell et al. 1988	Sweden	742	3,342	19	0	14	0	5	
Sirchia et al. 1991	Italy	780	5,200	52	0	50	0	1	
Total		6,560	36,176	559	0	507	32	11	1

them out as possible vectors. Since the manufacturer of the implicated AHF supplied 90% of the AHF used in Italy, an epidemiological investigation which uses a control group matched for age and severity of clotting-factor defect is extremely difficult. Nonetheless, a recently reported case-control study is compatible with the conclusion of product involvement (39), and this seems reasonable given the number of patients involved and the geographic spread of the disease. (In contrast, a measurement of seroprevalence of IgG anti-HAV amongst haemophiliacs in southern Italy showed that patients treated with SD-AHF, SD-treated prothrombin complex concentrate, or pasteurized factor AHF were essentially all identical (60-70% seropositive) and not different from the general population (40)). However, given the number of lots implicated and the use of U.S. plasma, it seems unlikely that plasma was the primary vector. One reasonable alternative was the process water, which was derived from a local well and purified by reverse osmosis. Before use, it was neither distilled nor heated at 80°C. A study has been initiated with Dr. Robert Purcell and with the co-operation of the manufacturer to determine if infectious HAV or HAV genomic sequences could be found in some of the implicated lots of AHF. A preliminary report of the results was given recently. Although HAV was found to be present by PCR in at least one lot of product, infectious HAV was not found on injection of chimpanzees (40). Preliminary sequence analysis indicates that this strain of HAV is homologous to strains more typically found in Italy than in the United States (41).

Seventeen cases (nine icteric) of HAV transmission amongst haemophilia A patients in Ireland have recently been noted (42). Locally collected plasma was shipped to a different fractionator than described above; however, AHF was prepared by the same process method. Ireland was experiencing an outbreak of hepatitis A at the time the plasma was collected, and all cases could be traced to a single plasma pool (43).

Thirteen cases of hepatitis A amongst recipients of AHF concentrates in Germany have been described, and an international team has recently been formed to participate in the investigation. All the implicated product was produced by the same methods as described above. Hepatitis A did not occur in haemophiliacs treated with other AHF products. Even though extensive studies are only now beginning, several facts have emerged. The first four cases occurred at the same clinic in 1988 within a six-week period. Since these cases did not have a lot of AHF in common and were removed from subsequent cases by at least 15 months, it seems reasonable to conclude that these were probably the result of community transmission. Examination of four of the implicated AHF lots by PCR revealed that two were positive; however, most recently it was shown that the genomic sequence identified in the lot of AHF differed from that found in the infected patient.

Infectivity analysis is pending; clearly, final conclusions must await completion of the investigation; nonetheless, interesting, but as yet unevaluated, hypotheses have been advanced to explain why HAV transmissions might be occurring now although they did not occur previously, even before the use of viral inactivation methods. One hypothesis is that improved hygiene in many countries, including Italy, has caused a shift in the age of community exposure from the young to the blood donor population, thus increasing the HAV risk of blood and blood products. A second hypothesis is that improved hygiene has resulted in a lower level of neutralizing antibody present on the pooling of plasma units. A third hypothesis

is that neutralizing antibody present in the intermediate purity products in common use before 1989 has been removed by the more intensive purification schemes now employed. Such antibody could serve to neutralize virus that might be present occasionally. A variation on this idea is that when transfused, this antibody afforded passive immunity to the recipient, protecting against community-derived HAV infections. A fourth hypothesis is that use of plasma collected in regions or countries without a sophisticated regulatory system, such as possibly East Germany, might have contributed to the German outbreak. A fifth hypothesis, now ruled out (44), is that the ion-exchange column used in purifying the AHF in question serves to concentrate HAV, releasing it into the AHF eluate from the same batch or in subsequent batches. This might help to explain why hepatitis A has not been observed among haemophilia B patients treated with prothrombin-complex concentrates or amongst haemophilia A patients treated with SD-AHF prepared by other purification methods. Of course, any combination of factors, including those listed above, might be operating simultaneously.

Several solutions have been proposed, the most immediate of which would be the vaccination of all non-immune haemophiliacs with the now available hepatitis A vaccine, or passive immunization with normal immune globulin. This approach seems reasonable since we cannot be sure that any of the coagulation factor concentrates in use today would be safe, given the resistance of HAV to SD and the relative heat stability of this virus. A second approach would be to combine SD treatment with a second method validated to inactivate or remove HAV and other non-enveloped viruses. For example, virus removal may be higher with the current monoclonal antibody purification schemes than with ion-exchange chromatography, virus removing filters are now becoming available, and the heating of SD products in the lyophilized state at 100°C has been recommended (45). Validation of HAV killing and protein compatibility are necessary. A third approach would be to improve the quality of plasma by ceasing to collect in regions endemic for hepatitis A or during a hepatitis A outbreak.

CONCLUSION

The SD method of virus inactivation has been validated extensively with regard to virus killing and protein compatibility. SD-treated products would appear to enjoy a high margin of safety with regard to all major blood-borne viruses, including HBV, HCV, and HIV. The SD method can be inserted readily into virtually any purification scheme, providing predictable and effective virus killing. The high specificity of reaction derives from its mechanism of action, being directed against the lipid coat of enveloped viruses. Non-enveloped viruses, should they be present, will not be inactivated; thus, protection against these viruses needs to come from other factors, e.g., donor selection, antibody neutralization, and/or complementary methods of viral removal and killing. Fortunately, in a transfusion setting and with products of cell culture, including monoclonal antibodies and recombinant DNA-derived products, non-enveloped viruses present little if any risk. Several recent outbreaks of hepatitis A in haemophiliacs in Europe treated with an AHF concentrate prepared using the SD method and the same ion-exchange chromatographic system requires additional investigation as to source of virus and reasons for transmission. These cases stand in contrast to the absence of icteric hepatitis A trans-

mission in the United States (46) or Japan (47) and to the historical hepatitis A safety exhibited by all blood products, including coagulation factor concentrates. Meanwhile, because of the uncertainty surrounding these transmissions, and the overall stability of HAV to killing, physicians should consider vaccination of susceptible haemophiliacs with one of the newly available HAV vaccines or passive immunization with immune serum globulin.

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UNITED STATES OF AMERICA

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION

AND RESEARCH

OFFICE OF BLOOD RESEARCH AND REVIEW

BLOOD PRODUCTS ADVISORY COMMITTEE

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53RD MEETING

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THURSDAY, DECEMBER 12, 1996

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The meeting was held in the Versailles Rooms I and II, Holiday Inn Bethesda, 8120 Wisconsin Avenue, Bethesda, Maryland 20814, at 8:00 a.m., Scott N. Swisher, M.D., FACP, Chairman, presiding.

PRESENT:

SCOTT N. SWISHER, M.D., FACP, Chairman
LINDA A. SMALLWOOD, PhD, Executive Secretary
CHARLES S. AUGUST, M.D., Member
BENJAMIN CHENG, Member
CORBY S. DUBIN, Member
GARY ELLIOTT FRIEDLAENDER, M.D., Member
BLAINE F. HOLLINGER, M.D., Member
JERRY A. HOLMBERG, PhD, Member
CAROL K. KASPER, M.D., Member
SUSAN F. LEITMAN, M.D., Member
WILLIAM J. MARTONE, M.D., Member
KENRAD E. NELSON, M.D., Member

PRESENT: (continued)

BEATRICE Y. PIERCE, R.N., MSN, Member
JANE A. PILIAVIN, PhD, Member
JOEL I. VERTER, PhD, Member
REVEREND VIOLET C. LITTLE, Non-voting Consumer Representative
PAUL N. MESS, M.D., Non-voting Industry Representative
PAUL R. McCURDY, M.D., Temporary Voting Member

JONG LEE, M.D., FDA
THOMAS LYNCH, PhD, FDA
TOBY SILVERMAN, M.D., FDA
EDWARD TABOR, M.D., FDA
MARK WEINSTEIN, PhD, FDA

PUBLIC COMMENTERS:

VAL BIAS
DR. RICHARD DAVY
FRED FELDMAN, PhD
CHRISTOPHER LAMB
M. SUE PRESTON
DONALD TANKERSLEY

SPONSOR REPRESENTATIVES:

SUZANNE COURTER, BSN, Genetics Institute, Inc.
EDWARD FRITSCH, PhD, Genetics Institute, Inc.
JOHN PETRICCIANI, M.D., Genetics Institute, Inc.
HOWARD GROSSBERG, M.D., Vitex
BERNARD HOROWITZ, PhD, Vitex
THOMAS R. OSTERMUELLER, Vitex

ALSO PRESENT:

JIM REILLY
CURT SCRIBNER, M.D.

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MR. TANKERSLEY: Dr. Swisher and members

of the Advisory Committee, my name is Donald Tankersley. I'm a technical consultant for the fractionation industry. I am not being compensated for my presentation nor for its preparation.

Restricting the maximum pool size for plasma fractionation may adversely affect the safety of plasma derivatives. Lynch, et al., in an article published in the journal Transfusion earlier this year, demonstrated by mathematical modeling that decreasing the size of plasma pools would, at best, decrease the risk of exposure to an etiologic agent in proportion to the reduction in pool size. However, for all but the rarest of infectious agents, this risk of exposure would be only minimally affected even by large reductions in manufacturing scale.

The article further pointed out that risk of exposure does not equate to risk of infection, which is always less. Other factors may result in a decreased risk of infection with increasing pool size.

These factors include: Neutralization and/or complexing of infectious agents by specific antibodies; neutralization or complexing by fortuitous nonspecific antibodies; and the dilution of infectious agents to noninfectious levels.

The most notable evidence that

neutralizing antibodies play a beneficial role in the safety of plasma derivatives lies in the historical safety of immune globulin with respect to hepatitis B transmission. Before the application of viral inactivation methods, this safety derived at least in part from the almost universal presence of anti-HBs in the product.

One lot of immune globulin, produced before third generation testing for HBsAg was mandated, was implicated in the clinical transmission of hepatitis B. This lot was subsequently shown to be infectious in a chimpanzee, and was found to contain practically no anti-HBs.

Although the level of anti-HBs in plasma pools may depend upon numerous factors, its presence or absence is dependent upon pool size. Simply by chance, antibodies of low prevalence in the donor population may be totally absent in small pools. For example, some lots of specific immune globulin preparations have been found to contain no anti-HBs.

Although current viral inactivation processes are very effective against hepatitis B, many have only limited usefulness against other infectious agents. Freedom from transmission by immune globulin preparations of parvo and hepatitis A, for example, is

likely due to the presence of neutralizing antibodies against these agents.

Decreasing the pool size may have a substantial impact upon the presence of antibodies to other known or unknown etiologic agents occurring at low prevalence in the donor population. A decrease in pool size would have the greatest impact upon the safety of immunoglobulins, but it cannot be assumed that other products, including coagulation factors, might not also be adversely affected.

Antibodies need not be neutralizing in order to affect the safety of plasma derivatives. The experience with anti-HCV screening has taught that, merely by complexing with virus, antibodies can have a substantial effect upon partitioning.

The second factor that needs to be considered,, the potential for neutralization of infectious agents by fortuitous antibodies, is far too complex to explain in detail in the time allotted. The overall concept is that antibodies to any agent are capable of being generated by the immune repertoire.

The production in quantity, by an individual, of multiple antibodies -- to different epitopes present on a virus or other etiologic agent

normally requires previous exposure to that agent or to a vaccine. However, an individual may produce antibodies directed to a different immunogen, which fortuitously cross-react to a particular epitope of an agent to which he has not been previously exposed.

Another individual may produce fortuitous antibodies to a different epitope. When one pools the antibody diversity from many thousands of donors, it is possible that the combined activity of many such fortuitous, cross-reacting antibodies can result in substantial complexing or neutralization of an infectious agent, even though none of the individual donors has active immunity to this agent.

The whole may be greater than the sum of its parts. This concept provides an explanation, for example, for the observation that immune globulin preparations frequently test reactive for anti-HCV, even though derived from individual units which are non-reactive by the same test.

Finally, the effect of dilution of an agent by the pooling process should be considered. I would just like to offer one example of how diminishing the size of plasma pools might adversely affect safety.

There is no evidence that Creutzfeldt-

Jakob disease has ever been transmitted by plasma derivatives, even though it is a statistical certainty that many lots of plasma derivatives derived from the plasma of donors subsequently succumbing to CJD have been produced, distributed and used.

The reasons for this lack of transmission are unknown, but it may be related to the low level of infectivity in plasma from a CJD victim, coupled with a further greater than ten-thousand-fold dilution of this infectivity in a plasma pool. Can we be confident that limiting the extent of this dilution by mandating a diminished pool size will still afford safe products?

In conclusion, although pooling plasma from many donors may increase the risk of exposure to infectious agents, pooling may actually decrease the risk of infection. This decreased risk of infection may be facilitated by dilution and complexing or neutralization of infectious agents by either specific or fortuitous antibodies present in large pools.

Unless it can be proven that substantial decreases in pool size would significantly improve safety mandating such a decrease does not seem warranted. Thank you for your attention.

CHAIRMAN SWISHER: Are there questions for

Mr. Tankersley? Thank you.

The next reserved speaker is Chris Lamb from the American Red Cross.

MR. LAMB: Good afternoon. My name is Christopher Lamb, and I'm the Vice President of Plasma Operations for the American Red Cross. Thank you for the opportunity to speak before the Blood Products Advisory Committee regarding the size of plasma pools used in the manufacture of plasma derivative products.

My responsibilities include the supervision of the manufacture and distribution of plasma derivative products from plasma recovered from volunteer whole blood donations collected by the American Red Cross. Parenthetically, over 99% of Red Cross plasma is recovered from whole blood collections. A small amount is source plasma.

By way of background, the American Red Cross does not own or operate a manufacturing facility for plasma derivative products, but has entered into several contract manufacturing agreements which allow for the manufacture of products exclusively from American Red Cross voluntary plasma under private label.

Red Cross recommends that any discussion regarding plasma pool size should take into account

Mr. SHAYS. Let me just say for the record so we don't have to dispute these issues, this committee believes and the Department—HHS believes that we have the safest blood supply in the world. This is not an issue. It's also not an issue of whether you all have made significant strides—the industry has made significant strides in the last few years to improve the quality of the blood supply. I think we all acknowledge that in the 1980's, we just weren't as vigilant as we should be. HHS acknowledges it wasn't as vigilant as it should be in monitoring the safety of the blood supply.

The issue that I am—I know, Mr. Chairman, you have some questions. And you've given the option of whether I can just go first and so on, because I need to leave. I have an appointment at 1, and Mr. Snowbarger will come in to Chair the rest of the hearing. So I just want to focus in on a few issues, and I don't need a response from everyone if you all agree with the response. I want to just focus in on the size of the pools, the lot size, and I just want to understand certain elements of it a little better.

First off, I will say to you that both this committee and the FDA were surprised by focusing on the pool size and then not focusing in on what I guess becomes the lot size, when you take different pools and then you combine these pools, and you end up with a lot size that can get into the hundreds of thousands. And so I'm just going to say to you that that was a surprise to this committee. And we need to understand its implications.

First, explain to me, and I'll go with you, Mr. Reilly, the difficulty of having a smaller pool size, whether it's 30,000 or 15,000. Why are smaller pool sizes costly and reduce supply?

Mr. REILLY. Let me break that into two or three parts perhaps, and then my colleagues might want to chime in with some additional detail.

First of all, with respect to costs, let me say that the Association has not dealt with the cost question at all. We've tried to deal with the size of the pools as purely a safety question. There can be little question that there is a cost. At this point, we simply have not made an assessment of that issue.

What we've tried to do and what we've said to you today is that we have determined, after a great deal of discussion, that there are opportunities for us to immediately reduce the size of the pool off the highs that have been reported. That means at least a 40 percent reduction off the hundred thousand.

So what we're saying is, clearly, we are going to set a ceiling then at 60,000 for the major products that we have described for you. So I think that is an important point that I want to reiterate.

I want to make a second point, and that is throughout the discussion of pool size, there has been confusion about the unit of measure. That confusion has led to, I think, a sense that perhaps people are not being forthright in the discussion or perhaps are being deceptive. I would like to try to dispel that because I genuinely do not believe that that's true.

Mr. SHAYS. You have your own agenda right now, but that's not my question. And with all due respect, my question is just trying to understand why, when you reduce the pool size, we create inefficiencies and we reduce supply of product. That's what I'm trying to understand.

Mr. REILLY. Let me try to answer that in two ways.

Mr. SHAYS. And someone else can. Maybe I should go to a manufacturer.

Mr. REILLY. If you are looking for a highly technical answer, then I would defer to a manufacturer. Maybe I can answer it on the basis of the comment that you made earlier, which was to say you wanted to come back and look at the chart we provided. So perhaps if we look at the chart again, I can make one point, and then I can allow one of my colleagues to come back with more of a technical response.

Can we put that chart back up on the easel?

When we made this chart, the goal was to make an assessment of the consequence if we went to full implementation of the FDA's proposal of 15,000 liters which had been suggested in the previous BPPA. Each of the companies internally looked at their systems, and they acknowledged to each other in the conversations that we had of trying to assess what was—what opportunities were here, that within their systems, their systems were very different. And those differences made simple explanations very hard.

What we attempted to do was have each manufacturer look at their own systems and assess what would happen if they fully applied the proposal that FDA had put forth at a previous BPPA meeting. We then turned that data over to an outside third party along with the consumption data for 1996 and said, if, in 1996, the full implications of this standard were to be applied with all of the elements that the individual companies have to take into consideration, what would be the consequence on supply? And the consequence, as this chart shows, is that there are substantial, then, reductions over what——

Mr. SHAYS. Mr. Reilly, I don't mean to be rude, but I'm with you there. I just want to know why. Just tell me why. That's all I want to know.

Dr. Feldman.

Mr. FELDMAN. Maybe I could have a try at it. I think there are a number of questions, Mr. Chairman, and I'll try to focus on trying to answer what's the problem in making it smaller, maybe making it more often, what are the difficulties there. I think there are potentially several different answers.

Now, one answer is it depends upon what range you talk about. If you're talking about from very large, like you quoted 400,000 down to 15,000, that's one set of answers. If you talk about making it small to try to improve the risk of potential exposure below that, there's another set of answers. But I've tried to detail——

Mr. SHAYS. You know what I'm going to do, I'm going to cancel my next meeting. I'm going to go to you, Mr. Burton. I'm sorry. I'm going to stay as long as I have to stay. Why don't you start, Mr. Burton.

Mr. BURTON. I won't take much time, Mr. Chairman. I was interested in the CJD issue. Before I get to that, though, is there any attempt being made to come up with synthetic supplies for these various problems for hemophiliacs or other diseases?

Mr. FOURNEL. Sure. There is a recombinant form of Factor VIII that is available.

Mr. BURTON. I can't hear you. I'm sorry.

Mr. FOURNEL. There is a recombinant form, recombinant DNA-derived form of Factor VIII, for example, and recently for Factor IX deficiency, that's available from several companies. So there are efforts where possible. But there are reasons why that can't be done for all products.

Mr. BURTON. With that shortfall that the chairman was just talking about, because of the pool size, is that something that can be overcome with the development of synthetic products?

Mr. FOURNEL. Potentially in some, by not all cases.

Mr. BURTON. How long would that take?

Mr. FOURNEL. Well, it's quite a long process, of course, to develop products. But, for example, a recombinant Factor VIII is available now in the United States from two or three companies. And it is, in fact, impacting the market significantly in terms of providing this therapy as an alternative to the plasma-derived form that you saw.

Mr. BURTON. So the problem that the chairman was talking about at some point in the future might very well be overcome——

Mr. FOURNEL. Potentially.

Mr. BURTON [continuing]. For that safety factor because of the synthetic products.

Mr. FOURNEL. For that case, yes.

Mr. BURTON. OK. Now, getting back to the CJD quickly. Is there any scientific evidence to support that conclusion that it's spread through blood transfusions?

Dr. GOMPERTS. May I answer that one? From the epidemiologic point of view, studies were already mentioned by—carried out through the American Red Cross and the Center for Disease Control. Studies in Australia, Germany, United Kingdom that have been completed as well as those that are ongoing showed that this agent, whatever it is, because we don't know what is causing this disease, is not being transmitted or cannot be detected to be transmitted through the blood supply or the products that we make. However, there are experiments that have started. They take many months, and some cases years, to do. And these experiments, the very first indication is that it is possible under some circumstances in mice or hamsters for transmission to occur.

Mr. BURTON. So it's not conclusive yet.

Dr. GOMPERTS. It will take some years to finalize.

Mr. BURTON. OK. Do scientists know how it's spread in humans? And is there any reliable way to diagnose it?

Dr. GOMPERTS. The methods of spreading have been documented to be, in my opinion, two forms. The one is through the food supply. And this has been documented through the unfortunate episodes in the United Kingdom and also in other countries in Europe, the "mad cow disease" situation.

Mr. BURTON. And the diagnosis?

Dr. GOMPERTS. I beg your pardon? And the diagnosis is made by a clinical evaluation. There is no laboratory test. And also at autopsy or biopsy of brain tissue.

Mr. BURTON. So it's mainly after the person has been——

Dr. GOMPERTS. Impacted by the disease, exactly.

Dr. DAVEY. If I may add, there's also some evidence that the disease—there is evidence that the disease has been transmitted by

transplantation of dura matter, which is a covering of brain, and as well as by human-derived pituitary growth hormone, which was used in the 1980's and is not used anymore. So those are risk factors for transmission of the disease.

Mr. BURTON. OK. Is there anything that the blood products industry can do to guard against the possibility that CJD is transmitted through blood products?

Dr. DAVEY. I think Dr. Gomperts has already touched on that—some of those issues, Mr. Burton.

In the Red Cross, we are very concerned about this issue; and we do, as I believe I outlined in my testimony, have several research projects under way. We are working with Dr. Brown at the NIH, looking at transmission studies to see how the agent is transmitted in an animal model.

We are also anxious to look at inactivation of the virus and whether or not it can be filtered out of blood and blood components, and we have active research with Dr. Robert Rohwer at the VA.

We also have epidemiological studies looking at recipients who have received products from donors subsequently diagnosed as CJD and have an extensive look-back study. Those data, as I mentioned, are encouraging and support the conclusion, I think, of the moment that we don't have good evidence that this disease is transmitted by transfusion.

Mr. BURTON. I don't mean to be redundant, because I know some of you probably covered some of this in testimony before I got here, so I apologize for that.

How many cases of CJD were reported in 1996 in the United States? And I am sure that doesn't compare at all hardly with England, for instance. But can you give me a number? I don't recall.

Dr. DAVEY. I can't give you the exact number for 1996, but the incidence has been very stable at one case per million per year. So we are experiencing about 250, 260 cases per year in the United States. That incidence has held steady since the disease was first described in the 1920's.

Mr. BURTON. And, I presume, that, in all of your views, that the industry is doing everything they can possibly to make sure that that is minimized and the public is protected?

Mr. REILLY. Well, what has been described is a variety of research initiatives that are under way to assess some of the unknown, but clearly that is what we are dealing with here, is a great deal of unknown. What we have learned over the years is that, in areas of the unknown, there is a pretty good consensus that says you incrementally deal with what you do know and find your opportunities.

What we are doing at the collection end is to impose donor criteria that allow us to identify potential donors who might have some risk and remove them from the donor population.

At the other end, in the product area, what we do is when, postdonation, we learn information that suggests that a donor was somehow at risk and not captured at the donation period, then we have been taking what I think most people would consider a very conservative strategy of retrieving product from the marketplace when those kinds of occurrences happen.

Mr. BURTON. Mr. Chairman, thank you very much for your hospitality. I appreciate it.

Mr. SHAYS. Any time. We appreciate your being here.

Mr. Towns.

Mr. TOWNS. Thank you very much, Mr. Chairman.

Could you help me very quickly? What is the distinction between pool size and lot size, as brief as possible? I need some help.

Mr. FELDMAN. Let me try, and I will try to be brief in my answer.

The pool size in its strict test definition is how many donors start the process going. But because of combinations of fractions in order to make sufficient quantity of drug, at the end of the process the pool size is represented in a final vial from the batch in terms of all of the donors that have been encountered by processing from the beginning.

The lot size, the batch, is the final product that is released for distribution, that has gone through a combination of steps along the way.

Mr. SHAYS. Could I just try to answer, and you tell me if I am right? You have pool A, pool B, and pool C. A lot can be a combination drawing from pool A, pool B and pool C.

Then you have this lot that you disseminate—you draw from all three different pools; and then, in effect, if you had a pool size of 50,000, you would have a pool size of 50,000 here and here. It comes down to one lot distribution combining these products, and you end up with 150,000 of donors to participant donors. Is that an accurate description?

Mr. FELDMAN. That is a good example of how a batch can be put together, yes. But the donors that contribute to that are a function of the fractions that are combined, as you have stated, and a function of the stabilizer that is added as well.

Mr. SHAYS. Still, if you have three pools combined into one lot, you add up all the donors to each pool.

Mr. FELDMAN. That is right.

Mr. FOURNEL. Don't forget, you can also have repeat donors. It doesn't mean you have 150,000 donors.

Mr. SHAYS. You would have some reduction factor, that is true.

Mr. TOWNS. That leads me to my next question. Would members of the industry be able to conduct a consumer-level recall program?

Dr. DAVEY. That is an important question, and I think we heard some very powerful reasons that we need to do better at notifying the patients when a recall is in effect.

In the Red Cross, we have been concerned about this because we feel the system is inadequate. I can review briefly what we do when a recall occurs.

We notify as quickly as possible the NHF, hemophilia treating centers, hospitals, other intermediate providers as quickly as possible with information about a recall. We also are very attentive to education programs for hemophilia treaters and their patients about recalls and what they mean.

We support the right of a patient to know as soon as possible when a recall takes place; and, therefore, we have supported regulations that would require intermediate distributors of our products

to record lot numbers. We feel that is an important step for us to trace the material to the end user.

So we feel that this is an important issue, and we need to do better.

Dr. GOMPERTS. Congressman Towns, the products that we are licensed to manufacture and market are prescription products, so that we are not permitted to know the end user. The physician writes the prescription for his or her patient. So the communication between my company and the specific end user, the patient, is not appropriate. It is not permitted.

The issue of recall through to the end user is an important one. This has to be addressed satisfactorily to the end user, ultimately the end user patient welfare, because that is what we are all about. So that activities are ongoing, communication is ongoing, in trying to resolve this issue; and these communications are between industry and between the FDA and also the representatives of the Hemophilia Foundation.

Mr. TOWNS. Mr. Chairman, if I am understanding this correctly—I really have a concern, because you are not telling me that you have any way to tell whose refrigerator this is in or whose house this is in. I think that is the bottom line; and I think that—as Dr. Davey indicated, I think that is where we have to go. I appreciate your comments, but that is a real concern. I think, Mr. Chairman, we need to look at this very carefully.

Yes, Mr. Reilly?

Mr. REILLY. I think we share that concern. You know—and I don't want to use this a lot—but we are in a very complicated area. We make a variety of products that are used by a diverse variety of patient populations.

In some circumstances, it is very practical for us to think we are going to be able to identify the groups who effectively represent those patient populations and know them quite well. In other cases, we distribute products in which that is not as easy to do.

For example, with our albumin product, which is used in burn treatment, it is frequently not very practical to get right to a user group who would represent that audience.

What the industry has attempted to do is we, in conversations with the Food and Drug Administration and the Blood Products Advisory Committee, recently acknowledged that we thought there were some things that could be done immediately or quickly without a lot of barriers toward improving the communication; and there were other things that perhaps needed more discussion and ultimately might need laws or regulations to deal with the barriers that are there.

So what we have suggested is that we would undertake the responsibility to create, within our group, a Web page that would be hyper-linked to a variety of different groups so we could improve or make a contribution toward improving information that flowed out to at least the well-defined populations of patients who use plasma products.

Beyond that, we would work and network with, if you will, those user groups so that we could identify specific parties, that we could ensure that the information got to them so that they, in turn, could ensure the information got to the constituents that they represent.

Beyond that, we have suggested to the FDA that, because there are very real legal privacy barriers that make notification directly to all patients difficult, that we encouraged FDA to consider convening some open public meeting where all those issues could be aired; and then we could determine which kinds of notification systems might be available and what regulatory or legislative barriers might exist to accomplish these.

So I think what we have tried to do is do this in two-steps: try to do those things we can do quickly, and then let's try to find out where the barriers are to a more substantial and comprehensive program.

Thank you.

Mr. TOWNS. Mr. Chairman, I am going to yield back. I feel I know what we need to do, so I am going to yield back.

Mr. SHAYS. Mr. Snowbarger.

Let me just say, we are going to go on for a bit. We are really nailed down on where we see our agreement and disagreement, so we have time.

Mr. SNOWBARGER. Thank you, Mr. Chairman.

As I hear more and more about this, I guess I am getting more and more confused, particularly about CJD. My understanding—and, again, I forget which witness mentioned it in the answer to Mr. Burton—was that we have not yet been able to isolate the factors that cause CJD, and yet we are performing experiments all over the world to see how we can pass it on. Somebody lost me somewhere.

You know, what kind of experiments are we doing—with what? To whom—when we don't know what factor it is that causes this particular disease? Anybody?

Dr. DAVEY. I will do my best. I am not in active research on this, Mr. Snowbarger, but I think you have hit a very important point, that the research that is being conducted now is hampered by the fact that we haven't identified the agent that actually causes CJD, and we have no tests for it.

We do know that there is a transmissible agent involved. This has been documented, because pituitary human-derived a growth hormone, dura mater and other means have transmitted the disease in very isolated circumstances.

So what we have tried to do——

Mr. SNOWBARGER. Excuse me. We have incidents of those transfers that are specific enough to know that that is the only factor that could be the explanation?

Dr. DAVEY. Yes, sir, that is correct. With the growth hormone and dura mater, that has been documented that that is the mode of transmission. So the experiments have to be done in an animal model using evidence of transmission without actually being able to identify or test for the agent itself. This complicates the research.

But I think Dr. Brown and Dr. Rohwer and Dr. Dronan at our lab are expert at working on some of these issues, and they are conducting experiments that I think can follow transmissibility in animals and can follow an activation process in animals without actually having a test for the agent itself.

Mr. SNOWBARGER. Yes?

Mr. FELDMAN. Could I answer that, Mr. Snowbarger? I think you have identified exactly the right questions that we are all trying to grapple with, in terms of how do you do experiments in this area to tell you some things so you can decide what to do with that.

Mr. SNOWBARGER. Very frankly, that is the reason for my question. We stand here prepared to tell you how you are supposed to operate your business, when if you don't understand what you are doing, I am not sure how we understand what you are doing. Therefore, I don't understand how we feel like we——

Mr. SHAYS. Would the gentleman yield?

The reason is that we had hearings in the previous Congress and we got into this whole issue of what happened in the 1980's. When you have hemophiliac patients who can tell you of the loss of their brothers and sisters and sons and parents and that half of the hemophiliac patients contracted AIDS, you begin to say, I am going to wake up, and I am not going to go on the assumption that, because I can't prove it, it doesn't equal no threat.

We went through that in AIDS. This committee is going through it right now with chemical exposure. We had the DOD tell us if our troops didn't fall on the spot, they weren't exposed to chemicals; therefore, there is no chemical threat. We are now learning that 90,000 of our troops and more were exposed to low-level exposure. DOD says low-level exposure doesn't equal chronic illness and death. That is not proven. We can't prove the opposite, but we can't prove that statement.

Therefore, we have to go under some assumptions that it may or may not be a problem. And the issue that this committee is interested in is partly because the consumers would like smaller lot sizes. They would like smaller pool sizes. They don't want to wait a long, long, long time to find out if we have a problem with a particular pool size. It is a fair request for them to make. And I am really happy you are here, because we need to have this kind of dialog.

But we haven't even scratched the surface of what you presented today, and we are going to try to find out where Mr. Snowbarger and I happen to agree.

If the pool size doesn't need to be larger, then why not make it smaller? And I want to have someone prove to me—and you can, because you are in the business—why the pool size has to be larger.

So, you know, we will come to some conclusion, and then we will realize where we have disagreements.

Mr. SNOWBARGER. I understand that, Mr. Chairman; and I agree with you that we ought to be attempting to find a good solution for consumers. But in the initial panel that we heard from, we heard—at my questioning, we heard that the ideal pool size is either one or infinite, because we think we can dilute the CJD. Nobody knows that either.

Mr. SHAYS. Would the gentleman yield? I think what we heard is we don't know.

Mr. SNOWBARGER. That is what I am trying to say. I don't know what infinite means, so we must not know. We know that if we can get the unit size down or the lot size down to one, then you can

know what with relative certainty. Obviously, that is not a practical solution either.

Mr. Reilly, just so that you have an opportunity, you had indicated in questioning from the chairman that there is some confusion about discussion on units. Would you like to pursue that and finish your answer?

Mr. REILLY. As the chairman has pointed out, this committee focused on pool size some time back. I think the debate has gone on for quite a while. Certainly it dates back to 1995.

Over the period of time, as the discussion has proceeded, the unit of measure has changed. We initially started talking about donations in the starting pool of an active ingredient and then progressively scaled that up. The discussion evolved to donors being the more appropriate unit of measure to decide what risk might exist or how to ameliorate the risk.

As we got into the question of donors, and as our people started looking at whether there were opportunities to do something, what became clear is that there was a wide range of practices; and when you look at FDA's testimony and discussions of how they would like to see the problem resolved, you find different numbers being used for different sets of circumstances.

For example, the 15,000 liter number is a number which doesn't take into account excipients. When you add excipients, which is a dilution we add to the product to stabilize it, it changes the numbers.

Our members looked at and determined they have a variety of practices that cause the numbers to move around.

As the discussion continues, it depends on where you walk into the conversation as to what you hear. I can't do anything, and I don't think our industry can do anything, about what happened in the past with this. But what we have attempted to do today in preparing our testimony and preparing our statement about where we can change the number is to try and not have that confusion persist past this point and talk in absolute donor exposure numbers. By taking into account all of the different issues that go into this and hope that if all of the other parties engaged in this debate look at it that way, we will have a less confusing conversation.

Mr. SNOWBARGER. Thank you.

Again, this kind of goes to my basic education in this whole area. When you are producing your products, you take this pool of donations, donated blood. Do you use that one pool for several different products, I presume?

Dr. GOMPERTS. Yes.

Mr. SNOWBARGER. Then are there different optimal pools, depending on the product that you are trying to produce? I saw heads shaking on the last one—I have to say that for the record—one shaking on this one.

Mr. REILLY. Let me start the answer, because I think the answer is multiple. It is complex.

The fact of the matter is that the variables are from company to company, product to product. So the answer that the Bayer representative would give for his constraints and his way of building his products would probably be different than the answer that the

Centeon representative would give you. That is where some of the confusion arises.

Dr. DAVEY. Just a point I would like to raise. I think in terms of the voluntarily donated recovered plasma, the issue may be a bit clearer, in that when we talk about the number of donations in a pool, it is a fairly clear number, because we don't have repeat donations essentially in the recovered plasma pool. Our donors donate every 8 weeks and no more frequently.

So when I outlined our efforts to reduce our pools to fewer than 60,000 donations, I think we can focus on that number. We have been successful with the Red Cross material in limiting our pools that we use to manufacture IVIg and AHF to fewer than 60,000 donations. We are not quite at 100 percent but we are well in the range. More than 90 percent of our pools now meet that criteria, and we intend to press on, especially with our albumin derivatives.

Mr. SNOWBARGER. And you feel fairly confident about that number. What tells you 60,000 is appropriate, as opposed to 30,000?

Mr. REILLY. The 60,000 number was arrived at through consultation with the experts in the companies with the first objective of trying to do something immediately—or rapidly, if not immediately. The criteria were related to where the opportunities today are that will allow us to make a change and come down to a number less than the highs that you heard, recognizing that there are barriers below some number. These barriers are: the need to reconstruct parts of the plant; the need to revalidate equipment; and, the need to engage in a variety of activities that are the result of changing the volumes, all of which require FDA approval. Any one of those things causes delays.

So what the companies concluded is, let's see where the opportunity is to set a precise number below which we assure you we will be. Then each of the companies individually will continue to examine that question, company by company, product by product, and engage in direct conversations with FDA about what other opportunities may exist beyond the 60,000 cap that we have agreed to.

Many of the companies, and I think it is reflected in their testimony, believe that today, in many of the cases, they are already there. So what we are really dealing with is the odd situation that is over the 60,000. We are committing to bring those down. Maybe some of the companies might want to comment on—with some of the detail.

Dr. GOMPERTS. I think part of the problem, in trying to answer your question and also Congressman Shays' question, is I don't believe there is any member of this panel who has sufficient experience and is qualified to talk about the reasons for these particular constraints, and that is the manufacturing constraints. I certainly am not.

But there are constraints as the volume and the particular components and the fractions are moved through the fractionation supply. There is equipment constraints. There is constraints right at the end, for example, in putting the product into the bottle and ensuring it dries properly. There are constraints in the equipment that purifies the specific products.

But I certainly don't have the expertise to provide the answers in depth.

Mr. SNOWBARGER. Mr. Chairman, I know you have been involved in conversation here, but I think that Dr. Gomperts made a very good point. That is, if we are trying to get a full understanding of pool size and its impact, particularly on supply ultimately, we don't have the engineers and the manufacturers before us to tell us what those constraints are.

As was indicated—I don't know if you want to restate the point you are trying to make, but there are constraints in the manufacturing process that make smaller amounts maybe less efficient. Maybe efficiency is what we are talking about.

Mr. SHAYS. You mean you all aren't prepared to talk about that?

Mr. SNOWBARGER. There are some.

Mr. FOURNEL. In my written testimony—I had to read through this so quickly, unfortunately—we tried to offer a very specific example for our product, and I talk about specific——

Mr. SHAYS. Hit the mic just a little.

Mr. FOURNEL. I talk about specific equipment constraints, so I can certainly refer you to that.

While I agree with Dr. Gomperts that I am, at least, not prepared to talk about every detail of processes, I think we can certainly provide testimony.

Mr. SHAYS. We are going to walk through that. I am going to be around for a while.

I want to understand the whole issue of the equipment, the dryer size, a little bit. I want to know if that is the nature of what you have established today or whether that is just inherent in the process to—generic need to have a certain size, or whether that is what the industry has now. I mean, we don't have many in the industry. How many players do we have?

Excuse me, are you done?

Mr. SNOWBARGER. Well, Dr. Feldman had a comment on the last question, and then I would be happy to yield back.

Mr. SHAYS. You don't have to yield back. Yes, sir?

Mr. FELDMAN. I would like to try to address the question again, and maybe an example would help clarify it.

I have a table that shows what happens with different vat sizes. It is table 9–B, if you could put it up. I don't want to overwhelm you with details, but sometimes it helps if you can see an example.

Mr. SHAYS. That did kind of overwhelm me. If you could simplify that, it would help.

Mr. FELDMAN. Most of the numbers we don't need to talk about, but we can talk about two things—the batch size in terms of volume, the top line, and it shows a range from 15,000 to 500. That is in liters.

Mr. SHAYS. These are different size vats.

Mr. FELDMAN. Different size final batches, this is everything contained in that. This is asking what can you do across the range.

The second line shows the number of donors contained in this process and each calculation from that volume. So for 15,000 for our process, going into the details of production and counting how many there were, it is in a range between 53,000 and 81, or it could be all the way down to 500 liters in this process. And looking

at the number of donors, there could be, even at 500 liters, a major reduction. There could still be 42,000 to 64,000 donors.

The reason is because as—even as we decrease the bath size, the product that is there, that factor has stabilizer added. That is albumin. It brings its own donors in. And unless we address that separately, even though we decrease the effect of principal, the fact is the donors are still there.

While we do that, because we are decreasing the volume, the quantity units we can make decreases. So what we can do to serve our patients drops. So that is not a very effective way of addressing that.

The question that we have asked is, without attempting to so radically redesign our plants, can we still address improvement?

So if you look in one column at a time—let me take the 15,000 liter column—the question is the donors per batch range, even at that fixed volume, can we address those independently of how big the vats are? And the answer is, yes, we can.

We can—in our case, because I don't know of the details of my colleagues' processes, we have asked what is in there that we can address to make the numbers smaller? And we have found ways to do that without having to go and ask for a whole new plant to be built. That is part of the initiatives I spoke to.

But I believe we can address those questions so that, even if there isn't agreement on does it matter in a safety perspective if we have a small batch or not, that we can still talk about improvement for improvement's sake; and I think we have all agreed that we can do that.

Mr. SHAYS. When I use the words "manufacturing economies of scale," I can view it two ways: I can view it just in your ability to produce enough product or I can view it in terms of cost.

Let's just make the assumption I mean it in terms of your ability to produce enough product. I want to go over the pluses and minuses of a large pool, and I want to see if we have some agreement on that.

One would be manufacturing economies of scale. I am just thinking of your ability to produce more in the same amount of time. There is the theoretic risk of dilution. There is the concept of what I gather was—dilution would be, in my judgment, you just spread it out. Ultimately, this one bad donor spread throughout the entire system, that donor no longer becomes a threat.

I look at naturalization as being kind of good cells battling to overcome bad cells. You probably use another word than "cells", and it probably offends you, but you get my point.

Then the concept that we had enhanced genetic diversity. You needed—what—enough different antibodies. That is what I have down as plus.

Mr. FOURNEL. If I can make one comment, sir.

While I appreciate what you want to do, I wanted to add one thing to the list Dr. Zoon provided on the plus side, and that is product availability. I realize you just put that together with efficiency. But I would suggest they are actually different. I think most of my colleagues——

Mr. SHAYS. Instead of my saying manufacturing economies of scales, you want me to think in terms of that, in terms of cost. I

will do that. And then you want me to think of product availability as a separate one. That is a fair way to do it. That is a good way.

Now on the other side you have one—and maybe there are ways to get around this. One is the recalls are much more difficult, as there is so much product to recall and different kinds of products. So that would be one.

Then, there is the concept of what I call spread. One bad donor can harm not just 1 person, but can harm 10 or 20. In other words, it can spread out. Using this contaminated supply, one bad donor results in many people being infected.

There is the concept of exposure. The opposite of naturalization is enhancement. In other words, naturalization, where the good cells overcome the bad, you could have the opposite, the bad cells overcome the good. Would that be the concept of enhancement?

Mr. SNOWBARGER. I think the word Dr. Zoon used was neutralization.

Mr. SHAYS. Excuse me, that is what it was—neutralization. Thank you.

Mr. FOURNEL. If I can suggest—the issue of neutralization is because we know that individuals may have a preexisting antibody to the very infectious agent that another donor might——

Mr. SHAYS. She basically acknowledged it is more proven that you have neutralization than enhancement. I accept that.

And then the big kind of scary thing is you would have an epidemic. In other words, you just simply don't know of something now and then you discover it, and you discover it in a large pool rather than a small pool.

Now, what would I add on the negative side? Anything else?

Mr. FOURNEL. I just had one comment to her negatives.

Mr. SHAYS. All right, I am encouraging that.

Mr. FOURNEL. The issue of bigger recalls or withdrawals assumes something—it is a little technical, but the repeat rate of the donors contributing to the product pools is an important factor. That is, the more times that an individual donor contributes to a pool, then the reduction in pool size really starts to be mitigated by the fact that that donor is represented in——

Mr. SHAYS. Are you tying to tell me one bad donor in five pools is no different than the five pools being in one lot?

Mr. FOURNEL. Than having that same donor—all units from that donor going into one large pool.

Mr. SHAYS. OK, that is fair.

Tell me, when you have said that you could easily reduce from 100 to 65, explain to me, that you could reduce the lot size a bit—I want to do it this way. You could reduce the donor exposure to a user from 100 to, say, 65. Why is that the case? What makes that—am I correct? Has that been said?

Mr. REILLY. That is correct.

Mr. SHAYS. And does the industry agree? You are the representative of the industry.

Mr. REILLY. What the industry has said is when they examined their current practices they saw an opportunity to move rapidly to move to a limit of 60. In the course of the discussion, what they also acknowledged is that, for each company, the method that they

would use to accomplish that would vary, depending upon their unique situation.

Dr. Feldman, in his presentation, catalogued for you a number of things that his company believed that they would engage in to accomplish that goal.

In Dr. Davey's testimony, he talked about being able to be at that same goal as well. In the Red Cross's case, they would use a different menu of options in order to get there.

Mr. SHAYS. Anybody else want to respond? Is it your testimony that, basically, you can reduce the donor exposure to a user—let me back up a second, just because I made assumptions that I shouldn't assume. Let's take each of you.

What is your basic donor to user size? Let's just start with you, Ms. Preston.

Ms. PRESTON. I need a little help with "donor to user size." I think we have provided it to the FDA, and I think even in our written testimony——

Mr. SHAYS. Is this proprietary information?

Ms. PRESTON. To some extent, yes, I think all of us have——

Mr. SHAYS. Then I am going to take an average.

Ms. PRESTON. An average? Excuse me?

Mr. SHAYS. Would average help us out here?

Ms. PRESTON. Maybe by product would be better. I think for some of our products we are at 30,000, 40,000. Some of our products are higher than that, at somewhere in 80—or 60 or 70 or 80, and we have to look at our practices.

There is a menu. I agree with what has been said. There are ways we can look at reducing the donor exposure in a given lot.

Mr. SHAYS. Dr. Feldman.

Mr. FELDMAN. I am not clear exactly what you are asking me in terms of donor to user.

Mr. SHAYS. I guess what I am trying to do is get around the difference between pool and lot size. When we had this hearing 2 years ago—and my understanding of the FDA, in private conversations as well as their public statement, was that they, too, were surprised by the number of the lot size when we go in the 400,000 range. That blew their mind, and it blows our mind.

It just tells me that wasn't something the industry was eager to share with us 2 years ago, or I guess you could say, well, we just didn't know what question to ask. But, we are trying to get a handle on it.

Ms. PRESTON. If I could, please, I used to work at the FDA from 1980 to 1988.

Mr. SHAYS. So it is your fault.

Ms. PRESTON. It is all my fault. No. So I wasn't surprised at 100,000. I wasn't surprised at less.

Mr. SHAYS. They weren't surprised at 100,000. They were surprised when it got over 100,000.

Ms. PRESTON. I think part of it is people being familiar with how we do our batches, and people who have been out to our facilities do see our batch records and see how many donors it does take for a given set of products. So I think, depending on who at the FDA was looking at things, some may have seen where we were and others may not have.

Mr. SHAYS. They didn't know the answer to our question when they asked the question. They had to go out and find out. That tells us they were surprised.

Yes, sir?

Mr. FOURNEL. Can I just address this specific question?

Mr. SHAYS. I am going to come back to you.

Mr. FOURNEL. I don't know the statistics that you have from the other companies, but I do know what we provided to the FDA, and I believe we were responsible for at least one of the 400,000 number you are speaking of, if there is more than one.

At the risk of confusing the issue more, I want you to recall that there are two sources of plasma they were talking about. One is source plasma and one is recovered. And recovered plasma is generally much smaller volume, as has been explained earlier.

In our case, the product in question that had the 400,000 donor exposure was all derived from recovered plasma, so it represented a factor, as we have been talking about, the fact that we had so much less plasma per donation, that, in fact, it represented 400,000 donors.

To explain again, in other words——

Mr. SHAYS. I am fine with that, and you just triggered another question.

Dr. Feldman.

Mr. FELDMAN. I guess what I want to say is I don't want to add additional confusion, but even within one manufacturer and within one product there is variation in how many donors there are.

Mr. SHAYS. Give me your high and give me your low.

Mr. FELDMAN. So what I want to say is the initiative that the IPPIA spoke of is to adopt a ceiling to limit what the high is and to reduce the variation.

Mr. SHAYS. Dr. Feldman, I agree with Vince in that, if we don't have to limit the top end, why should we? You all are saying you can go from 100 to 65. You seem to be comfortable to describe that and that you can do it.

In each case, I want to know your highest level; and I want to know your lowest level. That is what we are going to do. That is not a hard question. Is it a hard question?

Mr. FELDMAN. Yes.

Mr. SHAYS. Why?

Mr. FELDMAN. Because we have to know what terms you want us to include in there. Is it with or without the albumin stabilizer? Is it the high end of our range for that?

We have been asked different questions. Sometimes it includes that, sometimes it hasn't.

Mr. SHAYS. OK. Give me two choices. What else?

Mr. FELDMAN. Let's include it, and let's include the most number of donors that there could be in a batch.

Mr. SHAYS. What I am trying to avoid is I am trying to avoid you all having to come back again. Maybe that is impossible. But, as it stands now, you are raising a lot more questions; and we will just be back and just try to iron it out. If you can try to help me out here, this won't have to be where you have to keep coming back.

Mr. FELDMAN. I really want to answer your question.

Mr. SHAYS. OK.

Mr. FELDMAN. For our different products, for our Factor VIII, for our Factor IX, for our albumin, for our IVIg, we have a different total number of donors.

For our Factor VIII, we have numbers in excess of 60,000, but believe we can come down significantly below that.

Mr. SHAYS. Dr. Feldman, "in excess" is 68 or is it 200,000?

Mr. FELDMAN. Up to 94,000, 94–95, including the albumin stabilizer and not taking credit for repeat donors.

Mr. SHAYS. I understand about the repeat donors. Believe me, I understand that.

Mr. FELDMAN. If I take credit for repeat donors and can demonstrate that, then that 95,000 number comes down to 63.

Mr. SHAYS. What does albumin take it down to?

Mr. FELDMAN. Without taking into account the albumin, it is 21,000 donors. It is very different. So that is why I need to clarify the term.

For our Factor IX, and I think most of our Factor IX's, it is much lower. The worst case, taking into account stabilizers for us and a non-repeat donor rate, is around 28,000.

Ignoring the non-donor repeat rate, if we can verify that there are repeat donors in there, the 28,000 in our case becomes 18,000. We are not talking about 400,000 at all. For our albumin, we are talking about a range of 20 to 30,000 for us.

For our IVIg, our numbers are higher. Without a repeat rate and with albumin accounting for the donors for the albumin, it can be 150,000. If I can verify the repeat rate, that number drops to 100,000, roughly; and if I don't take into account the albumin, it is 63. So that is the range of donors.

Mr. SHAYS. OK. The highest number you gave was 150; and you said if you could take in repeat, it would be 100?

Mr. FELDMAN. Right.

Mr. SHAYS. Ms. Preston.

Thank you, Dr. Feldman.

Ms. PRESTON. For Factor VIII—and again this is using sort of the same analogy—right now it is around 22,000 to 28,000 donors in the lot. When we add albumin, that puts it up significantly with another 46,000 to 52,000 donors there. And we can do a similar type of calculation with repeat donors also, but I think that needs to be verified and validated.

With some of our other products, such as albumin, we are somewhere higher than that. Albumin ranges from around 6,000 to 102,000, depending on whether it is 5 percent or 25 percent. IVIg, 75,000 to 125,000. But, again, we can get to the 60,000 ceiling for those.

Mr. SHAYS. OK. Dr. Gomperts.

Dr. GOMPERTS. Mr. Chairman, I don't have the specific data for my organization with me. The numbers are approximately the same as to my colleagues on the left.

Mr. SHAYS. OK. When you get back, if they are higher, we would request that you would contact the committee.

Dr. GOMPERTS. I will do that.

Mr. SHAYS. We are making a sense that under oath you are telling us they are the same; and if you find they are higher, then we would like you to notify us.

Dr. GOMPERTS. Sure.

Mr. SHAYS. Mr. Fournel.

Mr. FOURNEL. You want it by each product?

Mr. SHAYS. You can just do similar.

Mr. FOURNEL. I think, in most cases, it is similar. We, in fact, do believe we can document our repeat rate, so we would cite a lower number. But, without that repeat rate, the numbers are not too different from what you have heard.

Mr. SHAYS. Is the number higher than 150 in any product?

Mr. FOURNEL. No.

Mr. SHAYS. OK. If you are pretty convinced that these numbers don't represent a threat to anyone, why would you want to bring down the donor size? I am just going to ask you the reverse of what I have been asking you. Why would we do that? Why should you do that? I don't want you to do anything you shouldn't do.

Ms. PRESTON. Can I answer?

Mr. SHAYS. Yes.

Ms. PRESTON. I think when we look at our practices it is a good way of being more consistent. So, in that sense, for us it is a way of adding consistency, which is part of good manufacturing practices. So that is one way of looking at it.

It doesn't mean that there won't be some minimal effect on supply as some of the partial lots that we might have used would not be utilized under the proposed scenario of 60,000.

Mr. SHAYS. OK. Dr. Feldman? I mean, why bother? Why not just continue the way you are doing it?

Mr. FELDMAN. First of all, most of the batches we make aren't in this large size range. Most of our batches are lower than this. And we provided numbers——

Mr. SHAYS. Dr. Feldman, is your testimony that, like the others, you are going to reduce your donor size, your pool size?

Mr. FELDMAN. Unless instructed not to, we are planning to.

Mr. SHAYS. You are planning to. Why?

Mr. FELDMAN. We are planning to decrease the variation in the batch size range. We don't need to operate at this range and still put product out. There is no benefit to us in operating at a high end range like that. If we can operate in a narrower range without providing these numbers of donors, there is no reason for us to continue that.

Mr. SHAYS. So your testimony is you can reduce your donor size, your pool size, without changing significantly your supply side; and so your testimony is there is no reason to have a higher donor size if you don't need to? Is that what you are saying to us?

Mr. FELDMAN. I gave you three sets of ranges of numbers. On the high end, we believe that we can address those and bring them down to the lower range of variation. If we have agreement with FDA that we are not impacting any of our validation data or quality assurance, we are prepared to go ahead and do it.

Mr. SHAYS. I know you are prepared to do it. I want to know why you would do it.

Mr. FELDMAN. To decrease the variation. As my colleague said, because part of GMP says that process is under control. We would also like to have less variation batch to batch, just to have more control on it.

Mr. SHAYS. Dr. Gomperts.

Dr. GOMPERTS. As I see it today, the issue of batch size, pool size, does not impact the safety of our products. There is growing pressure from this committee and also the FDA. We have heard certain numbers, there is debate going on. It is important that we look at our processes to determine what the impact of reducing batch size will be, and the proposal that is put forward has indicated to us that the impact on supply will not be substantial.

Mr. SHAYS. Dr. Fournel.

Mr. FOURNEL. I would more or less concur with my colleagues. The only point I would want to make is that I think it is intuitively apparent that having 400,000 donors to a single lot of product is probably not a good idea. In fact, in a very unfortunate sequence of timing, that very lot is implicated or that very material is implicated in a withdrawal that we are having today associated with CJD in potential six lots of our Prolastin product.

So it is clear, with the CJD case in particular, having lots of that size or donor exposure of that size is probably contributing to the withdrawals that we have certainly experienced. In fact, all of our withdrawals——

Mr. SHAYS. So when you get up to 400,000, you are basically relating it to the whole issue of recall.

Mr. FOURNEL. I was trying to relate it to the issue of safety insofar as the donor exposure at that level would seem to—clearly, it is associated with the higher incidence of recall, because that has been our experience. So I think there is some rationale for reducing from those kinds of numbers.

I think the problem is, when we get below the 100,000 range or the 60,000 range, it becomes a much more difficult argument to have; and there are many, many factors that impact that argument. I think, as everyone said here today, the real reason we can all sign on to the 60,000 limit is we think it will improve our manufacturing processes more than necessarily impact the safety of the products.

Mr. SHAYS. One of the whole issues was availability of product, and the other issue is economy of scales. In the short range, there are economies of scale. Excuse me, are there economies of scale the larger the batch or the larger the donor pool? I am sorry.

Mr. FOURNEL. To some extent. Maybe if I can use the Prolastin example that I have in my testimony——

Mr. SHAYS. Can you talk louder?

Mr. FOURNEL. If I can use the Prolastin example I have in my written testimony, perhaps that would help. What ultimately constrains or sets the size of our batches is the size of our freeze dryer. We want to fill our freeze dryers completely. They are operated 7 days a week, 24 hours a day, in order to provide this product. So the size of that freeze dryer is what sets the basic size of our batches or our lots.

That being said, it turns out, for the reasons again that I have discussed in my written testimony and will not go through, that a

donor exposure of 60,000 using source plasma is what we need in order to get batches that would fill that freeze dryer and enable us to make the most product available with the equipment that we have.

Mr. SHAYS. OK. Explain to me why you take different pools and combine them? There has got to be a reason why you do that.

Mr. FOURNEL. Because the freeze dryer holds a certain number of vials. Let's say 5,000. I don't know the exact number.

Mr. SHAYS. So it is basically determined by the freeze dryer?

Mr. FOURNEL. That is right. At this level, at this particular example, that is the case.

Mr. SHAYS. In your judgment, are there medical reasons to have larger pool size, in excess of 60,000? Are there health reasons that you would want a pool size larger than 60, a donor-to-user size of larger than 60?

Mr. FOURNEL. Apart from the arguments that Dr. Cunningham-Rundles mentioned earlier, no, I can't see a medical reason.

Mr. SHAYS. Refresh me what her reason was again?

Mr. FOURNEL. Diversity in the spectrum of antibodies that are provided——

Mr. SHAYS. Antibodies. Is that the key issue of why you would want a larger lot size? I am talking about what we can agree on and what is proven. We were given these lists of what is being studied and looked at, but haven't been determined to be scientifically true, correct? I just want to know, are there scientific reasons and health reasons why you would want a smaller pool size, other than recall issues?

Mr. FOURNEL. Why you would want a smaller or larger?

Mr. SHAYS. Smaller. I am going to ask each of you.

Mr. FOURNEL. I thought you were asking me larger.

Mr. SHAYS. I asked larger first, and then I am going to go to smaller. You already have answered. You said none other than the issue of the antibodies. OK, yes, sir?

Dr. GOMPERTS. You mentioned the level of 60,000. Is that what you are focusing on?

Mr. SHAYS. Are there health reasons to have a larger donor to user pool size?

Mr. FELDMAN. I don't believe so.

Mr. SHAYS. Dr. Feldman? That is proven. I am not saying we may suspect or believe.

Mr. FELDMAN. Mr. Chairman, I know there are differences of opinion on this——

Mr. SHAYS. Fair enough.

Mr. FELDMAN [continuing]. And I have seen and I have heard cited, in fact, at the December 1996 Blood Product Advisory Committee from a Mr. Tankersly, also an expert in this area, that he believes there is an importance to pool size as a safety issue. I don't know that I can fully represent his opinion.

I think—in regard to our pools and 60,000, I think that we can operate within that range and that there aren't safety issues in operating below that. There are not.

Mr. SHAYS. The one thing I don't want to have happen is 10 years from now I look back on a hearing I had 10 years ago and

find out that we limited the pool size and then determine that that was a mistake, that you want a large pool size for whatever reason.

So I am putting you on record, and I am putting you on record to understand this, and then this is an issue we are going to proceed with. It is my sense that we simply don't know. We don't know if a larger pool size is better or not. We don't know if a smaller pool size is better or not, only based on intuition. We do know that availability of product would be affected, and that is fair. But I just want to know if you disagree.

Dr. DAVEY. Mr. Shays, if I could speak on that——

Mr. SHAYS. I am sorry, I didn't focus that way.

Dr. DAVEY. I think you summarized the issue very well, and I think the exercise we went through earlier in the day about listing pros and cons of a larger pool size was very instructive, and I think the answer is indeed that we don't know what an optimal pool size may be in a given situation.

But I think, at least in terms of the Red Cross position, we have taken the position that, given what we know, it is prudent to limit pool size where we can, to have a practical upper limit for different products that are manufactured from Red Cross-recovered plasma. Therefore, we have instructed our contract fractionator to have a 60,000 limit for IVIg and for our AHF and to use albumin stabilizer from that lot for that material. We feel that is prudent.

And where we can limit our pool sizes for albumin, we are going to move on that also.

But you are right. Pool size variation in the context of other issues and other measures that we can take to improve the safety of plasma derivatives and whole blood is just one of many issues and perhaps not the most important.

Mr. SHAYS. I think the only other issue, and then we can move forward—I am going to summarize, and it is basically repeating some of what you are saying.

What I am hearing the industry tell us, this committee, is that you are going to, in some cases, reduce the pool size. And in some cases where you have been using a figure of 100,000 down to 65,000, it is the testimony before this committee that there is no scientific knowledge that says a larger pool size is better or a smaller pool size is better in terms of the quality of the product, but that in some cases your pool size had been larger and you are going to bring them down a bit.

It is the testimony of this chairman that we are not putting—we do not both publicly or privately seek to have an artificial number, because we don't know what that number is.

It is the testimony of this chairman as well, though, that we were led to believe the pool size was much smaller. Given how we found out and the surprise notwithstanding, Ms. Preston, your comment of the FDA as not being able to get a handle on that figure, on that number soon enough, we felt that there was just simply not a candid dialog between the industry and Congress in terms of what that number was. We were surprised by it, and it raises a real concern.

There is always going to be a concern on the part of Congress that economies of scale, not availability of product, can sometimes dictate what the private sector will do. And that is one of the im-

portant rolls that Congress plays, is to say, is there an economy of scale coming in here to the detriment of public health? And we are going to ask questions to determine that, and this is one area that we will pursue a bit because we don't pretend to have this knowledge now.

The area, though, that I feel very important to end up with is what do we do for the consumers in terms of recall, particularly a recall of 400,000. I understand your testimony is that is unusual, correct?

Mr. FOURNEL. No, sir. The situation for us with Prolastin and the reason I used it as an example in my testimony is that we are the only supplier currently in the United States of this product; and this patient population has a very desperate need for this product, not just on a one-time basis but on a regular repeat basis. We have been doing everything we can to provide as much product as we can to this patient population, and that includes the purchase of intermediate fractions from other manufacturers that we can use in our process.

Mr. SHAYS. So that increases the donor size?

Mr. FOURNEL. That is what happened. Because we, as a commercial operation, only use source plasma for our pooling efforts. But in order to again augment the supply of Prolastin we purchased so-called 41 intermediate that was made from recovered plasma to use in the manufacture of the Prolastin product. When we combined those intermediates, all these recovered plasma intermediates, that is how we get up to the very high numbers because the numbers we have for Prolastin using source plasma are in the 60,000 to 100,000 range in general.

So the point I was getting at is that, because of the use of recovered plasma, we do have cases where we have these very high donor numbers. However, because all of our experience with CJD withdrawals with respect to Prolastin have been because of the use of recovered plasma intermediates, effective June of this year we discontinued procurement of these intermediates. So we no longer use them.

Mr. SHAYS. Just as a segue into this point before I talk about recall, there has got to be a tremendous economic incentive not to make your pool size too large, because when you do have recalls, I would think it would be quite expensive.

Mr. FOURNEL. Well, again, sir, understanding that a 400,000 donor recovered plasma number, you can equate to 100,000 donor source plasma.

Mr. SHAYS. OK.

Mr. FOURNEL. In other words, they—because the donor, the volume of the donation is so much smaller, recovered plasma—so it doesn't mean the lot is four times bigger.

Mr. SHAYS. Fair enough.

Mr. FOURNEL. So the same size lot—is the same size final product content.

Mr. SHAYS. Is it true that, as alluded to by one of the consumers who spoke to us, that you have had a significant number of recalls? Have your recalls become greater than in the past? I would like to know with each of your companies. Do you have more recalls today

than you had a few years ago? That may be just that we are just being more vigilant.

Mr. FOURNEL. Again, I'm sorry. As I put in my written testimony, seven of nine. Now, I have to say 8 of 10 recalls that we have had in the last 2 years have been because of CJD. So, yes, we have had more.

Mr. SHAYS. OK. CJD has been the reason why you have had the——

Mr. FOURNEL. That's the vast majority.

Mr. SHAYS. Not any of any of the other factors that in the past might have been the problem.

Mr. FOURNEL. I'm saying that we might have had two recalls that are associated with GMP issues, but that's more or less the nature of the business for us.

Mr. SHAYS. Dr. Gomperts.

Dr. GOMPERTS. The majority of our recalls have been CJD related.

Mr. SHAYS. How many have you had in the last year?

Dr. GOMPERTS. In the last 6 months, there have been five.

Mr. SHAYS. In the last year?

Dr. GOMPERTS. I can't tell you. Probably double.

Mr. SHAYS. OK. Dr. Feldman.

Mr. FELDMAN. Centeon has not had a recall for CJD. But we have had recalls for GMP-associated issues. We've recalled albumin last year. And we also had precautionary recalls for a Factor VIII and Factor IX. They were small recalls.

Mr. SHAYS. So during a 12-month period, the last one you could state to us, what would be the number of recalls?

Mr. FELDMAN. I'm not sure, maybe four or five.

Mr. SHAYS. OK. If it is larger than that, you will notify the committee.

Mr. FELDMAN. Yes.

Mr. SHAYS. Thank you.

[The information referred to follows:]

Fred Feldman, PhD
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August 5, 1997

The Honorable Christopher Shays
Chairman
Subcommittee on Human Resources
Committee on Government Reform and Oversight
The U.S. House of Representatives
Washington, DC 20515

Dear Chairman Shays:

Thank you for the opportunity to testify at the hearing regarding plasma pool sizes on Thursday, July 31. I hope that Centeon's presentation was helpful to the Committee.

I would also like to utilize this opportunity to further respond to the question you posed concerning the number of recalls Centeon has conducted over the past twelve months. Centeon has conducted six recalls during this period of time, with three of these related to our recall of Albuminar® last fall.

I hope this information is helpful.

Sincerely,

Fred Feldman, Ph.D.
Vice President
Preclinical Research and Development/
Biotech Evaluation

cc: Mr. Larry Halloran
Ms. Anne Marie Finley

Mr. SHAYS. Ms. Preston.

Ms. PRESTON. Alpha has not had any recalls for CJD as of yet. That doesn't mean that in the future we won't. We've had one recall of our intravenous immunoglobulin because this particular lot was associated with a higher rate of adverse events than we had experienced, so we took a precautionary action and recalled that. That—those are—I think that is the only recalls we had in 1997. And in 1996, we had, I don't know the exact number of recalls related to Hepatitis A.

Mr. SHAYS. So the total amount in the 12-month period?

Ms. PRESTON. I would say less than five.

Mr. SHAYS. OK.

Ms. PRESTON. But I will check on that and make sure.

Mr. SHAYS. If it is different than that or higher.

Ms. PRESTON. Yes, sir.

Dr. DAVEY. We, Mr. Chairman, and the Red Cross have definitely noted an increase in the number of CJD recalls since August 1995. And we think some of that reason is because, at that point, the FDA recommended that we begin asking our donors questions about family exposure to CJD. We're receiving a lot more information from our donors. As a matter of fact, 85 percent of our recalls are because of postdonation information we get from our donors. And so this is impacted on the number of our recalls.

Mr. SHAYS. OK.

Is it fair to say that most of the recalls 5 years ago would have not been because of CJD, it would have been for other reasons? And my assumption is that some of the reasons you have had in the past have been dealt with. Some of the—there have been improvements in your process that have resulted in your not having a need to have as many recalls for some of these other reasons.

Dr. DAVEY. I would think that's fair. I think recalls are always instructive because they indicate an issue that needs to be addressed, whether it's an issue in postdonation information that perhaps we're not eliciting properly or questions could be answered better or perhaps it's an issue in the way we handle producing or manufacturing our components. So recalls are instructive. And I think we've learned from them.

Mr. SHAYS. Let me just take the last question. If I were a user of blood products on a continual basis, I would become pretty well informed. And I would think, and maybe this is happening, that every manufacturer would be able to have a number I could call. I could literally call that number, hit whatever the batch number is. I don't—I've never seen how you would identify it, but is it a batch—what is it? It is a lot number. And I would be able to hit that lot number. And so, for instance, Mr. Feldman, when I called your company up, I could hit that lot number and it would tell me the status, that there is no problem with this lot number or that there is because of so and so. Does that happen?

Mr. FELDMAN. I'm not aware that we have something like that. But a system like that could be considered, yes.

Mr. SHAYS. Well, it is my understanding that you do not have to tell the end user of the product directly. It would be rather impractical to track down the end user, correct? You could contact where you sell it, but not the end user. And so then the question I would

have is, what are you all doing to make it easier for the buyer do that? I mean, on either the Internet or on the telephone? Do any of you do it? Yes, Mr. Reilly.

Mr. REILLY. Well, the association is in the process of initiating a project that is aimed in that direction. It's not quite as comprehensive or user-friendly as the proposal that you just made, clearly, but it has two elements to it. First, it is to create an association Web page to provide easier access to the withdrawal information that is available. And, second, to engage in a dialog and create a network with the major patient groups that have organized programs so that we can have a way to more rapidly disseminate information. It's not quite as elaborate as what you just proposed, but it's a step in that direction.

Mr. SHAYS. Well, why would that be elaborate? That to me wouldn't seem like a difficult thing at all. You just have a number—maybe I have a false impression. There aren't that many manufacturers; are there? Are we talking about hundreds or are we talking about a handful? This is it; isn't it? So it would seem to me—and so wouldn't every blood product that I have identify one of your companies?

Dr. GOMPERTS. True.

Mr. SHAYS. Would it identify the Red Cross? Would it identify it? It would. So I could—yes, sir.

Dr. GOMPERTS. I'm not quite sure what you're saying, but I think what, and correct me if I'm wrong, what you're asking is if a consumer of one of our products had used a particular lot——

Mr. SHAYS. Not had used or is planning to use. I look at it, and I want to check before I——

Dr. GOMPERTS. There is a question around that particular product and that particular lot. And if that individual has such a question, certainly there is customer service——

Mr. SHAYS. OK. Dr. Gomperts, I would like you to think about this. If I were a hemophiliac patient, and I had this blood product that I was using, and it had a particular identification number on it, I would want to just be able to call up your company. I would want to see the phone number on the bottle maybe. And I would call that company up, I hit these numbers, and I would get a readout. It said this product is good to use; there has been no recall of this product. And it just strikes me that, since you are not required to go and ultimately contact the end user, at least make it easier for the end user to contact you.

Dr. GOMPERTS. I believe we have such a system in place.

Mr. SHAYS. Well, if you end up calling someone who refers to you someone else, that wouldn't be very friendly, but if you do, that is good.

Dr. GOMPERTS. They come to my desk.

Mr. SHAYS. Let me say I believe you do or know you do is a difference. Describe to me how the system works, then.

Dr. GOMPERTS. I can give you—this occurs on a daily basis.

Mr. SHAYS. OK. Let me just say to you I don't want to be—I don't want to take a cheap shot.

Dr. GOMPERTS. Yes.

Mr. SHAYS. But if I were a hemophiliac patient, I would like to think that your company or any of the other companies would have

a system that you were so well aware of that you could just tell me chapter and verse how it worked. And maybe—yes, Mr. Reilly.

Mr. REILLY. Let me just make two observations and then a pledge.

First of all, what you have proposed has to do with providing an opportunity for recipients of our products across the full spectrum of all products available. What we've been discussing is an effort by the industry to try and enhance the communication where there is a product with a question specifically. I think those are really two different things. But what I do pledge to you on behalf of the industry, is that we will examine the question you raised and determine whether we can do something positive that is more responsive.

Mr. SHAYS. I think that that would be helpful. Because, what we may have ended up in this hearing is no real answer about donor size. So I am saying to you I am not sure how this committee is going to go in that area. But if, at the very least, we can't say a large donor size or smaller donor size is preferable, I would say we would say smaller is preferable where practical as long as we don't negatively impact the availability of the product.

It seems to me that one of the outcomes of this hearing may be that, at the very least, to the consumers who use your product, they should feel very comfortable in using your product. And while they are using this product, there isn't a letter on the way telling them not to use the product.

And given the number of recalls that you have said, they are not—it is not one every 3 years, it is something that happens. It would seem to me a very logical way to proceed.

So maybe one of the outcomes of this hearing will be that you will focus a little more attention on that. And I will say to you that this committee, and before we draft our report, will want to know what you are doing. And if you are doing something that we think is meeting the consumer, we are going to make sure that we publicize it and congratulate you for it.

Is there any final comment?

Mr. Davey, I kind of left you out and yet you are probably—Dr. Davey. I want to make you a mister, and I want to make Mr. Reilly a doctor.

Dr. DAVEY. I would just like to comment, Mr. Shays, I think your idea is excellent. And it actually was proposed by one of my colleagues, Dr. Peter Page, at the Blood Product Advisory Committee hearing a year, year and a half ago. And I think it's time we look at this again. I think it's an excellent idea. I want to compliment you. We thought about that before.

Mr. SHAYS. Let me end by apologizing to you, Mr. Reilly, and you, Dr. Feldman, because I think it was very unrealistic of me to think that I could have a few questions and then leave. So I apologize for my impatience and your trying to respond to my questions. I appreciate your being here and thank you.

I call this meeting to a close.

[Whereupon, at 2:27 p.m., the subcommittee was adjourned.]

[Additional information submitted for the hearing record follows:]

IMMUNE DEFICIENCY FOUNDATION

The National Organization Devoted To Research And Education For The Primary Immune Deficiency Diseases.

Kathy Miles Crews is a member of the national Board of Trustees for the Immune Deficiency Foundation. She is also the President of the Texas Chapter of the Immune Deficiency Foundation. She has been an active spokesperson for many years to benefit patients and families affected by primary immunodeficiencies. Mrs. Crews son Clayton was born with X-Linked Agammaglobulinemia. Her brothers Stephen and David are also affected with the same primary immunodeficiency. Their medical therapy includes bimonthly infusions of intravenous immungloubulin replacement therapy.

Mrs. Crews has been outspoken on the issue of Inactivated Polio Vaccine vs. The Oral Polio Vaccine. The CDC's recommended change in the polio vaccine schedule was quickly incorporated into the professional education programs she directs through her local chapter. The goal of the information provided by the Texas chapter is to assist pediatricians with making early diagnosis of primary immunodeficiency diseases.

Mrs. Crews professional background is in mortgage finance. Her husband Larry is President of an oil field company.

Mrs. Crews currently resides in the Houston area.

Thank you for the opportunity to submit written testimony to this Committee on behalf of the Immune Deficiency Foundation, my son Clayton, and my brothers who are immune deficient. My name is Kathy Miles Crews, I am a member of the Immune Deficiency Foundation Board of Trustees and the President of the Texas Chapter of the Immune Deficiency Foundation.

Mine is a typical American family, at least until one day in 1963, when our family was asked to line up with our entire community for a modern miracle cure for polio. We all took just a sugar cube with a medicine that was designed to eradicate the deadly disease. That day changed our family dramatically. Within two weeks, David my younger brother was paralyzed from the waist down. To the astonishment of the medical community, David had developed polio from the vaccine designed to prevent the disease. The result of David's vaccine associated paralytic polio was the diagnosis that he and my two other brothers suffered from X-linked Agammaglobulinemia a primary immunodeficiency (PID), affecting males.

The diagnosis was made in time to spare my younger brother, Stephen from receiving the vaccine. My older brother Dennis died as the result of complications of his primary immunodeficiency. At the time of his death, my brother Dennis, suffered from a brain tumor that robbed him of his sight.

As a female, I was spared this particular disorder, although many women are affected by the over 50 primary immune deficiency diseases. However, my brothers continued to suffer recurring infections that often required hospitalizations. The only treatment available to them at the time was the painful intramuscular injections of gammaglobulin. By 1980 our family was delighted to learn that gammaglobulin had become available intravenously, resulting in a higher, and less painful dosage. With the administration of IGIV my brothers received more antibodies, protecting them from a wider spectrum of disease and improving the quality of their lives. We were thus spared the frequent trips to the hospital, and became a relatively normal family once again.

Watching my brothers suffer with chronic illness, I never considered the possibility of having my own children. The doctors suspected that the PID in our family was genetically linked. However, with the advent of IGIV I found myself rethinking the possibility of starting a family of my own. It was not established that I was a carrier of this genetic disorder.

I married and with great anticipation my first son Cody was born healthy, four years later my son Clayton was born.

Early blood tests given to Clayton indicated that he had also been spared primary immunodeficiency. When he fell seriously ill at six months of age further testing revealed that he was indeed immune deficient. At eight months of age my baby began IGIV therapy. IGIV has allowed Clayton to grow into a normal healthy teenager. My brother Stephen has gone

on to become an allergist and immunologist. In his practice he treats other patients with primary immunodeficiencies.

IGIV has been instrumental in helping our family live a normal life, free from the fear of constant, recurring, and life threatening illness. However, in 1994 we learned that the medication used to keep us healthy had developed serious problems. Hepatitis C had been transmitted through the use of IGIV. We were not able to adequately obtain lot numbers associated with the Hepatitis C recalls. To this day my family is not sure of the lots affected with Hepatitis C virus and which of these lots we may have received. Our only recourse has been expensive and ongoing diagnostic testing. My son and siblings have had tests on their liver functions and ultimately PCR testing. So far they have tested negative, however this may not always be the case, and their health continues to be monitored. Our family situation is more fortunate then some others who are not able to afford this type of health surveillance.

At this juncture, our family along with thousands of others became proactive in issues related to blood safety. Concerns over recalls, withdrawals and notification became a paramount concern. We began to routinely record the lot numbers of the infusions we received. I would also like to mention the voluntary withdrawal of products containing donors who are at increased risk for CJD. The guidance offered by the FDA and the decision by industry to withdraw these products from the market place, strikes terror in the hearts of patients. Although, it has not been proven that CJD is a blood born pathogen, once the decision is made to remove CJD tainted product, the consumer/patient and their physicians need to be notified immediately to avoid being infused with these lots. We also became alarmed over the "off label" usage of IGIV, as we feared shortages associated with products being withdrawn.

Our story, and countless others, serve to illustrate the effectiveness of IGIV therapy. Our bimonthly infusions, keep us healthy and remain necessary for the entirety of our lives. The safety of the blood supply affects my family several times a month as my loved ones sit with an IV in their arms and are infused with the blood of many donors. Any changes made in the collection, manufacture, and distribution of these products **directly impacts my family**.

As a parent and sibling, I would urge this Committee to consider the perspective of immune deficient patients carefully before entertaining recommendations that will affect the monthly medical therapy of some 20,000 to 30,000 patients.. Arbitrary changes in such a successful therapy would be tantamount to repeating the Tuskogee experiment. It is essential that a representative from the immune deficient patient population sit on the current blood safety advisory committees, so that the considerations of the growing number of patients who are consumers of IGIV can be voiced and heard

Thank you for the opportunity to express my concerns.

IMMUNE DEFICIENCY FOUNDATION

The National Organization Devoted To Research And Education For The Primary Immune Deficiency Diseases.

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CREUTZFELDT-JAkOB DISEASE
MODIFICATION OF FDA RECOMENDATIONS

THURSDAY JULY 31, 1997

Chairman Shays & Distinguished Members,

The following letter was addressed to FDA staff from the Committee Of Ten Thousand regarding proposed changes in FDA policy regarding CJD and plasma derivative products. The current proposed change in the CJD policy illuminates a number of issues that we believe are indicative of what continues to be wrong with the regulation of the blood/blood products industry. We hope the issues articulated will help illuminate COTT's concerns for the Subcommittee:

We have given lengthy consideration to the proposed changes/modifications of FDA's December 1996 recommendations regarding plasma donors and single donor processed dura mater. We have reviewed the different options put forward by FDA staff and have difficulty with options 1-4 as well as with the overall presentation of the issues which are being defined and delineated in a very narrow fashion. From our perspective the discussion appears to be lacking in an analysis of the larger context. The issues are being presented here as if the landscape today is unchanged from that of July 1996. In fact some would argue that that body of knowledge regarding the threat posed by CJD does remain constant with what we knew at that time. However, we are seeing a growing and disturbing body of evidence that raises the real possibility CJD transmission through blood/blood products. While we still do not have a confirmed case of transmission through blood/blood products, we are seeing more indicators that necessitate both a much larger commitment of research dollars from government and industry as well as a heightened degree of caution in assessing the current regulatory landscape regarding CJD. Given the above, we do not find this the most opportune or advisable moment for modification of the current FDA policy and would therefore recommend the

2.

adoption of option 5; no change in current FDA policy.

When the Special Advisory Panel On CJD made its recommendations in July of 1996 the work of Dr. Brown at NIH had not been completed, the WHO recommendations had not been promulgated and we were unaware of the articles regarding transmission of CJD to any workers handling dura mater. { Lancet Vol. 341 pgs. 1-3} While clearly remaining unproven the blood/blood product transmission of CJD is more indicated today than it was in July of 1996.

Critical issues such as plasma pool size are not addressed in this discussion. This is an issue that clearly and directly impacts the discussion of CJD and this particular policy change. The use of such large pools {20, 000 donors or more per pool} certainly impacts the issue of both risk and the impact of potential product holds or quarantines. Given that the incidence of CJD in the population is roughly 1 case per million, significantly smaller pools would substantially reduce the impact of subsequently discovering that a donor had been at risk or had developed CJD. Since the amount of individual product units would be significantly lower the hold would therefore have a much smaller impact on product availability. It also would reduce the overall chances of a given pool containing the plasma of a donor who subsequently develops CJD. The question of pool size was not at all addressed in the discussion surrounding this proposed policy modification. This is another example of what we believe is the consistent addressing of critical safety issues in a very narrow fashion that fails to incorporate the larger picture or landscape of a given policy or issue.

The Committee Of Ten Thousand has, over the last five years, constantly been raising with FDA staff the issues surrounding plasma pool size. It is our contention that these very large pools are employed due to their efficiency only in terms of manufacturing economics of scale. They certainly do not represent the safest and most protective scale in terms of the users of plasma derivative products. In fact, plasma pool size is another instance where economic concerns, manufacturing economics of scale, have been allowed to take precedence over the question of what is the most desirable pool size in terms of maximizing the margin of safety for the users of plasma derivative products.

The question again becomes, lacking definitive conclusions, what is an acceptable risk for the users of blood/blood products and how much is the margin of safety to be adjusted in the name of availability and supply. These are the critical questions to be answered when considering the proposed modification of FDA policy. Unfortunately, the issue of recall, withdrawal, quarantine and hold becomes more difficult when the manufacturers are not forthcoming with the necessary data regarding the impact of a given policy like the one adopted by the CJD Advisory Panel. Consumers were subjected

to sometimes dire predictions about the potentially critical shortages that would be the result of the new policy when it was adopted in 1996. We were told that if this continues, "you guys will never have enough factor to meet your needs" rather than a factual and substantiated analysis of what the impact would be in a variety of situations involving the adopted policy. How are we all to make intelligent and well balanced decisions about the question of acceptable risks and availability if we have nothing more than alarmist rhetoric to work with. This is part of the context that COTT believes is not being addressed here.

This modification if adopted will allow, at a minimum, Baxter and the American Red Cross to immediately release holds on a very large number of units we are being told. It is also important to note that this is occurring in the context of Baxter having to initiate a number of product withdrawals and recalls this year alone. These actions have seriously impacted a large range of Baxter/Hyland's plasma derivative products such as Albumin, AHF, Immune-globulins IV, Plasma Protein Fraction and others. This series of recalls emanating from Baxter has impacted a wide range of the company's plasma products and certainly must be having a significant impact on product availability as well as the Baxter's economic picture. What role do these issues play in the current request for policy modification and how do we asses the request for policy change in this larger context?

In order to reach intelligent and well balanced decisions regarding both safety and availability we must have the data necessary to inform those decisions. As we have repeatedly stated this has not been, and is not, the case and we again find ourselves confronting an important decision with serious implications while not possessing the data to give us a clear understanding of the implications of each option and the overall landscape regarding the potential impact of any one option. This is a situation that must change if we are to make intelligent decision that maximize the margin of safety for the users of blood/blood products.

Again, we at COTT are struck by the FDA's unwillingness to exercise the power invested in it by the Congress. Any data relating to decisions about safety and availability should be, and from our perspective can be, demanded by FDA. Why are we consistently working with limited knowledge of certain facts and data sets that are imperative to understanding the implications of any given policy option or change. This continues to maker no sense if our goal is to reform the regulatory structure and maximize our margin of safety.

Another issue that seriously impacts the range of safety discussions is the question of strong regulatory authority versus this concept of "building consensus" which we hear so often when having discussion at the FDA. It is our contention that at a given point on the time line consensus building became synonymous with self-regulation and a lack of real oversight by those at the FDA tasked with regulating the blood/blood products industry. We

certainly support the concept of close cooperation between industry, government and consumers, however, without strong regulatory independence and enforcement what we will be left with is a repeat performance of the 1980's.

If we all share the goal of creating the safest blood supply possible then why do we continue to be plagued by the same problems and barriers to a clear understanding of any given problem.

For COTT, the question continues to be, will the next emergent threat devastate an entire community before we effectively respond or will we have created the kind of cooperation and strong regulatory climate that will result in the impact being relatively small and contained? At this time the jury remains in deliberation as to the answer to this critical question. We as the recipients of the last regulatory failure are certainly committed to changes necessary to prevent a repeat performance of the 1980's.

COTT's leadership continues to call for the creation of a new climate of cooperation between industry, government and the user community. It is our contention that this is the only effective approach to maximizing the protection of the health and safety for the millions of yearly users of blood/blood products.

Yours In The Public Interest,

Committee Of Ten Thousand-Science & Medicine Working Group

Corey S. Dubin
President COTT

Dr. Rich Colvin
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