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21 CFR Parts 606, 610, and 640

Biological Products; Blood and Blood Derivatives; Implementation of Efficacy Review; Proposed Rule

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 606, 610, and 640

[Docket No. 82N-0163]

Biological Products; Blood and Blood Derivatives; Implementation of Efficacy Review

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: The Food and Drug Administration (FDA) is proposing to amend the biologics regulations in response to the report and recommendations of the Panel of Review of Blood and Blood Derivatives (the Panel). The proposed rules would affect blood banks, plasmapheresis establishments, and plasma derivative manufacturers. The Panel reviewed the safety, efficacy, and labeling of blood, blood components, blood derivatives, and related products. On the basis of the Panel's findings and recommendations, FDA is proposing to classify products in Category I (safe, effective, and not misbranded), Category II (unsafe, ineffective, or misbranded), and Category IIIB (off the market pending completion of studies permitting a determination of effectiveness). Products recommended for Category IIIA (formerly defined as on the market during further studies in support of effectiveness) will be reviewed by the Blood Products Advisory Committee for reclassification into Categories I or II.

DATES: Comments on the proposed classification of products into Category I and on proposed amendments to the biologics regulations should be submitted by March 24, 1986. Comments on the confidentiality of data submitted for review by the Panel should be submitted by January 23, 1986.

FDA proposes the following: (1) That any final rule based on this proposal become effective 60 days after the date the final rule is published in the **Federal Register**; (2) that amendments to a license application, other than labeling amendments, be submitted to FDA within 180 days after the date the final rule is published in the **Federal Register**; (3) that labeling requirements, other than the requirements in §§ 201.56 and 201.57 (21 CFR 201.56 and 201.57), become effective 12 months after the date the final rule is published in the **Federal Register**; and (4) that the labeling requirements in §§ 201.56 and 201.57 become effective 30 months after the date the final rule is published in the **Federal Register** (see § 201.59).

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ADDRESS: Written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Steven F. Falter, Office of Biologics Research and Review, Center for Drugs and Biologics (HFN-364), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-295-8046.

1. Background

In the **Federal Register** of February 13, 1973 (38 FR 4319), FDA issued § 601.25 (21 CFR 601.25) concerning procedures for the review of the safety, effectiveness, and labeling of biological products licensed prior to July 1, 1972. Under the panel assignments publish in the **Federal Register** of June 19, 1974 (39 FR 21176), the biological products reviewed were assigned to one of the following categories: (1) Bacterial vaccines and bacterial antigens with "no U.S. standard of potency," (2) bacterial vaccines and toxoids with standards of potency, (3) viral vaccines and rickettsial vaccines, (4) allergenic extracts, (5) skin test antigens, or (6) blood and blood derivatives.

Under § 601.25, FDA assigned responsibility for the initial review of each of the biological product categories to a separate independent advisory review panel consisting of qualified experts to ensure objectivity of the review and public confidence in the use of these products. Each panel was charged with preparing an advisory report to the Commissioner of Food and Drugs which was to: (1) Evaluate the safety and effectiveness of the biological product, (2) review the labeling of the biological product, and (3) advise the Commissioner on which biological products under review are safe, effective, and not misbranded. The advisory report includes a statement classifying products into one of three categories.

Category I designates those biological products determined by the Panel to be safe, effective, and not misbranded. The Panel's statement may include any condition relating to active components, labeling, tests required prior to release of batches, product standards, or other conditions necessary or appropriate for their safety and effectiveness.

Category II designates those biological products determined by the Panel to be unsafe, ineffective, or misbranded.

Category III designates those biological products determined by the Panel not to fall within either Category I or II on the basis of its conclusion that the available data are insufficient for classification and for which further testing is therefore required. Those biological products in Category III for which continued licensing, manufacturing, and marketing, during the period of further testing are recommended, are designated as Category IIIA. Those biological products in Category III for which the suspension of their license, pending submission of additional data, is recommended are designated as Category IIIB. The recommendations for either Category IIIA or IIIB are based on assessment of the present evidence of safety and effectiveness of the product and the potential benefits and risks likely to result from the continued use of the product for a limited period of time, while questions raised concerning the products are being resolved by further study.

In the **Federal Register** of December 13, 1974 (39 FR 43413), FDA requested data and information regarding blood, blood components, and blood derivatives.

Some concern has been expressed that confidential information submitted to FDA under § 601.25 will become

public information. Data and information submitted in response to the February 28, 1973, June 19, 1974, and December 13, 1974 notices and falling within the provisions of 5 U.S.C. 552(b), 18 U.S.C. 1905, or 21 U.S.C. 331(j) have been handled as confidential. However, with the publication of this proposed implementation and the Panel's findings, such data and information will, under § 601.25(b)(2), be made publicly available after January 24, 1986 and may be viewed at the Dockets Management Branch (address above), except to the extent that the person submitting the data and information demonstrates that it still falls within the confidentiality provisions of one or more of the above statutes. Accordingly, comments concerning confidentiality should be submitted by January 23, 1986.

The Panel appointed by FDA to review the data and information submitted and to prepare a report on the safety, effectiveness, and labeling of blood, blood components, blood derivatives, and related products included the following individuals:

Panel Chairman, Scott N. Swisher, M.D., Professor, Department of Medicine, Michigan State University, East Lansing, MI 48823;

Joseph R. Bove, M.D., Director, Blood Transfusion Service, Yale-New Haven Hospital, New Haven, CT 06510;

John A. Collins, M.D., Chairman, Department of Surgery, University Medical Center, Stanford, CA 94305;

William S. Howland, M.D., Chairman, Department of Anesthesiology, Memorial Hospital, New York, NY 10021;

Robert D. Langdell, M.D., Professor of Pathology, School of Medicine, University of North Carolina, Chapel Hill, NC 27514;

Jacob Nusbacher, M.D., Director, Rochester Regional Red Cross Program, Rochester, NY 14607;

Sherrill J. Slichter, M.D., Associate Director, Puget Sound Blood Center, University of Washington, Seattle, WA 98104.

The Panel was convened on June 26, 1975, in an organizational meeting. Working meetings were held: August 22–23, October 31–November 1, 1975; January 9–10, March 19–20, May 5–6, June 25, October 1–2, November 12–13, 1976; January 14–15, March 11–12, June 17–19, October 7–8, November 18–19, 1977; January 6–7, March 10–11, May 12–13, July 17–18, December 15–16, 1978; and November 15–16, 1979.

Two nonvoting liaison representatives served on the Panel. Louis M. Aledort, M.D., nominated by the National Hemophilia Foundation, served as the consumer representative. Margaret Hilgartner, M.D., or Robert Schwartz substituted for Dr. Aledort during his absences. John Adams, Ph.D., of the Pharmaceutical Manufacturers Association, nominated by a number of producers with products under review by the Panel, served as the industry representative. Karl Bambach, Ph.D., or Charles B. Cleveland substituted for Dr. Adams during his absences. Morris Schaeffer, M.D., Ph.D., participated in the Panel meetings in his capacity as Director of the Office of Scientific Advisors and Consultants, FDA. Clay Sisk, in the Office of Scientific Advisors and Consultants, FDA, served as Executive Secretary of the Panel.

In addition, the Panel considered the advice of the following consultants:

Edward H. Mealey, Ph.D.
Findlay E. Russell, M.D., Ph.D.
James T. Sgouris, Ph.D.

Over 290 persons attended one or more meetings of the Panel and many participated in the open discussions. Every person who requested an opportunity was heard by the Panel. The names of these persons are on file with the Dockets Management Branch as listed in the Summary Minutes of each meeting.

The Panel on Review of Blood and Blood Derivatives evaluated all data submitted for the following blood, blood derivatives, and other related products:

Licensed Biological Products Considered by the Panel[1]

Blood Bank Products
Stored Red Blood Cell Products (Includes Whole Blood (Human) ACD and CPD)
Whole Blood (Human) Heparin
Whole Blood (Human) Modified
Red Blood Cells (Human)
Red Blood Cells (Human) Frozen
Cryoprecipitated Antihemophilic Factor (Human)
Single Donor Plasma (Human)

Plasma Fractionation Products

Normal Serum Albumin (Human)
Plasma Protein Fraction (Human)
Antihemophilic Factor (Human)
Factor IX Complex (Human)
Fibrinogen (Human)
Fibrinolysin (Human)
Rh_o(D) Immune Globulin (Human)
Thrombin
Fibrinolysin and Desoxyribonuclease Combined (Bovine) (with and without Chloramphenicol)

In Vitro Diagnostics

Blood Group Diagnostics
Blood Grouping Serums
Reagent Red Cells
Anti-Human Serum
Hepatitis B Testing Reagents

Miscellaneous Products

Antivenins (three products)
Normal Horse Serum
Blood Group Substances A & B
Cobra Venom Products

Data submitted for these products are cited by Biologics Efficacy Review (BER) volume numbers. The volumes will be available in accordance with the provisions of 21 CFR 601.25(b)(2) at the Docket Management Branch (address above).

The following products were originally listed for Panel review in the December 13, 1974 notice, but are no longer licensed as biological products and are not reviewed in the Panel's Report:

Albumin Microspheres (Human)
Anti-Human Chorionic Gonadotropic Serum
Modified Plasma (Bovine)
Radioactive Albumin products
Russell Viper Venom

The advisory panel appointed to review data and information concerning the safety, effectiveness, and labeling of blood and blood products has completed its review and has submitted its report to the Commissioner. FDA announced the availability the Panel's report to the public in the Federal Register of November 21, 1980 (45 FR 77134). The Final Report of the Panel on Review of Blood and Blood Derivatives follows.

2. Introduction to Panel Report

The last decade has seen rapid progess in those areas of science basic to blood transfusion therapy. This has also been a decade of significant change in clinical practice, many changes have occurred as a result in the ways in which blood and blood products are employed as therapeutic agents.

A few examples can be cited. Our knowledge about the problem of post transfusion hepatitis has broadened greatly. Testing donors for the presence of the hepatitis B associated antigen has effected significant reduction of the incidence of this disorder following infusion of materials that potentially carry this agent. In turn, this has focused attention on the problem of so-called "non-A, non-B" hepatitis for which adequate testing procedures do not exist. The blood coagulation system and its complex interrelationships with the complement system and the kinin system has been further elucidated. Similiarly, clinical experiences with

[1] Only biological products that have been licensed prior to July 1, 1972, are reviewed in this report.

No Source Plasma

therapeutic preparations of procoagulant proteins has increase our understanding of their utility as well as limitations on that utility. We not have a much better understanding of the physiological role of albumin, coupled with greatly improved clinical management of shock and massive blood loss. Factors affecting oxygen transport by intact red cells have become known, and their clinical significance explored.

These and other developments indicate that a review of blood and blood products licensed in earlier times before our new knowledge was available is timely and in the public interest in assuring the safety and effectiveness of these materials. This review is the product of one of six advisory panels of FDA's Bureau of Biologics and is part of the general review of biological products licensed prior to July 1972 (see 21 CFR 601.25). (Note: Since the time of that Panel's submission of his report, FDA has been reorganized. The Bureau of Biologics is now the Office of Biologics Research and Review (OBRR), Center for Drugs and Biologics.)

The work of the Panel on Review of Blood and Blood Derivatives has occupied a stipulating and informative 3 years. At the outset, it seemed that the Panel's task would be an easy one. The Panel was to deal with a well developed, widely distributed, and accepted group of technologies, a number of familiar products and therapeutic materials in widespread use by most physicians in practice. As the work of the Panel progressed, a significantly different picture emerged. In some areas, particularly with older products, it appeared that highly defensible scientific information about safety and efficacy was scarce. In dealing with some of these problems, it became necessary to rely upon consensus based upon common experience. This experience does, however, point out that the standards which are now applied to justify the introduction of new products have evolved along with the scientific knowledge which makes new products possible. It would seem that the complexity of the regulatory process has grown no faster than has understanding of the biological and clinical ramifications related to the introduction of new products.

A second observation was quickly made by the Panel: There are many eminently researchable problems which relate to a wide variety of blood products that present the opportunity for further improvement and refinement of these products and their clinical utility. Much of the effort of the Panel has gone into attempts to define these problems in researchable terms and to direct them, where possible, to appropriate investigators and sources of support for the work. It is the strong feeling of the Panel that this aspect of its work may, in the long run, be the most rewarding in terms of improved safety and effectiveness of the products review.

This Panel has had to deal with a situation rather different from that which has confronted previous panels which have reviewed other biological therapeutic materials. The bulk of the products reviewed are actually produced widely throughout the country in installations which range from large centralized blood banks, through smaller units, down to the size of a blood bank supported by an individual hospital. There are no large industrial "manufacturers" whose submissions and protocols can be reviewed as the basis for safety and efficacy determinations for therapeutic materials produced by blood banks. Thus, in this area, a manufacturer-by-manufacturer review of the Panel of every blood bank product is manifestly impossible. Rather, the Panel has had to rely upon years of experience with these materials, publications in the literature, submissions of procedures and standards of large blood bank organizations, data available in license applications, generic procedures specified by good manufacturing practices and standards of accreditation as the basis upon which to make judgments about safety and effectiveness of those products. In those areas in which commercial manufacturers do operate, as in the case of plasma derivatives, the more typical review of submitted information has been carried out. Some products were therefore reviewed only generically and recommendations made accordingly, leaving the determination of compliance for each manufactured product to the Bureau of Biologics which evaluates each installation.

The Panel has also been impressed with the difficulties encountered in obtaining adequate clinical investigational data upon which certain decisions about safety and effectiveness depend. Frequently the inadequacy of data is quantitative; not enough observations have been made to permit an adequate judgment, although there may be nothing wrong with the data that have been obtained. This problem arises in evaluating materials which have limited or no commercial sponsorship. It is also encountered in circumstances where a commercial manufacturer is unable to recruit enough clinical investigational resources within reasonable cost boundaries to complete a clinical evaluation which has been started. These difficulties are at the heart of the problem of shortening the time required for approval of new products or modification of old ones. Thought should be given to organizing a national clinical investigation resource to solve this problem in a cost-effective way. The Panel has no recommendations in this regard, but it is impressed with the significance of this problem in the effort to provide timely responsive regulatory actions.

The Panel wishes to thank the many people who have supplied it with consultation, expert opinion, data, and statements of interest for its consideration of a wide range of problems. These people have been related to the major blood-banking organizations of this country, academic institutions, industry, and the staff of the Division of Blood and Blood Products in OBRR. Without the help of these people and organizations, the work of the Panel could not have been completed.

Finally, the Panel wishes to express its strong feeling that this one-time review, whereas it has certain values as it stands, will be primarily of value if it is kept current by an on-going dynamic process. The science which underlies blood transfusion is rapidly changing. The potential exists now for development of many new products from blood. Rapid changes in health care delivery practices will occur in the near future. These factors will make a continuing review of this area over some reasonably short cycle of great value in assuring that the regulatory process enhances and supports the delivery of health care by providing safe and effective blood and blood products in a timely fashion to those who use them. The Panel has viewed its responsibilities from this perspective and has diligently attempted to provide a starting point for future activities in this direction. There are a number of the major unresolved issues which the Panel has identified. In most instances, additional information, the product of specific research, will be needed to resolve the problems which remain. Where it has been necessary to make a decision to deal with a specific problem until adequate data become available, conservative positions have been taken which have a "fail safe" character with regard to at least the issue of safety. With regard to effectiveness of products in this category, it should be noted that the materials reviewed have been in

widespread use over a period of years, and effectiveness has been documented at least by common experience. Thus the issue of effectiveness has been a lesser consideration when adequate data are lacking for decisionmaking in these cases.

3. Stored Red Blood Cell Products, History of Blood Transfusion, Donor Standards, Testing Donor Blood, and Labeling

Generic Statement

1. *Introduction*—a. *History of blood transfusion.* Although occasional successful blood transfusions were given in the 19th century, little real progress could be made until two major problems, immunological donor-recipient incompatibility and blood storage without clotting, were solved. In 1900, Landsteiner (Refs. 1 and 2) described what we now know as the ABO blood groups, and in 1911 Ottenberg (Ref. 3) applied this discovery to the selection of blood for transfusion. Progress in delineating other erythrocyte antigens was slow. Although in 1927 Landsteiner and Levine described the MN and P systems (Ref. 4), it was not until the discovery of the Rh system by Landsteiner and Weiner (Ref. 5) and Levine and Stetson (Ref. 6) in the early 1940's that immunologically safe blood transfusion could be practiced. Equally important at this time was the development of the antiglobulin test by Coombs et al. (Ref. 7) for the detection of nonagglutinating red cell antibodies. In subsequent years, new erythrocyte antigens were discovered at a remarkable rate; more than 400 antigenic specificities have now been described.

After the discovery of the ABO blood group, direct transfusion, a surgical procedure in which the donor's artery and recipient's vein were temporarily anastomosed, became popular. This was followed by the introduction of syringes or pumps to convey blood from donor to recipient. Direct donor-recipient transfusion became the standard practice for many years. A major difficulty was the tendency of the blood to clot, and the only logical solution was to find a safe anticoagulant to prevent this complication.

The safety of citrate for intravenous administration was demonstrated by Hustin in 1914 (Ref. 8) and by Agote (Ref. 9) and Lewisohn (Ref. 10) a year later. Rous and Turner (Ref. 11) in 1916 showed the beneficial effect of added dextrose on red blood cell preservation. Many years later, Maizels (Ref. 12) demonstrated that dextrose exerted its favorable effect by providing a source of energy for the synthesis of organic phosphate compounds. The acidification of the anticoagulant-preservative solution was first studied by Loutit, et al. (Ref. 13) in 1943 to prevent caramelization of the dextrose solution during sterilization. Serendipitously, it was found that acidification also gave better red cell preservation as judged by post-transfusion survival. The original Anticoagulant Citrate Dextrose (ACD) solution, suggested by Loutit and Mollison (Ref. 14), in 1943, has been modified primarily to reduce its volume.

Stored blood was first used by Rous and Turner (Ref. 11) in 1916, and in 1917 Robertson (Ref. 15) introduced it for treating battlefield casualties. Transfusion of stored, anticoagulated blood became a reality when the first blood banks were organized in 1937 in Chicago and in 1938 in New York City. Forty-four years later, in 1982, an estimated 9,349,700 units of whole blood were collected in the United States by 5,400 blood banks and transfusion services (Ref. 16).

A major development in blood bank practice occurred in the mid 1960's with the introduction of plastic collection containers which have replaced glass bottles as the standard blood container. This made possible the separation of whole blood into various components in an integral, closed sterile system. An inevitable result of component production from whole blood is the availability of Red Blood Cells (Human) ("packed cells"). This product is now widely used when erythrocyte transfusion is indicated and currently accounts for about 40 percent of all such transfusions in the United States.

b. *Whole Blood (Human) ACD.* Evaluation of the efficacy of ACD anticoagulant-preservative has rested primarily on satisfactory clinical responses following transfusion and on studies of post-transfusion red blood cell viability and survival. The accepted survival standard requires that on the last storage date at least 70 percent of the red blood cells be viable for at least 24 hours after transfusion. Both whole blood and red blood cell concentrates collected into ACD solution and stored at 2 to 6 °C easily meet this standard (Refs. 13, 14, and 28). Post-transfusion survival is related to the maintenance of adenosine triphosphate (ATP) levels in erythrocytes (Refs. 22 and 30).

Optimum storage, however, should encompass both erythrocyte survival and appropriate function. The major erythrocyte function, delivery of oxygen to tissues, is related to many variables, including erythrocyte diphosphoglycerate (DPG) level (Refs. 33 and 34). Red cells depleted of DPG release oxygen to tissues less readily than those which have normal levels. When human blood is collected in ACD, the DPG levels fall precipitously to approximately one-third normal after 1 week of storage (Ref. 35 and 241). However, the clinical significance of transfusion with DPG-depleted red cells is unclear.

c. *Whole Blood (Human) CPD.* In 1957 Gibson et al. (Refs. 36) published studies on modified ACD solutions buffered by phosphate to higher pH levels. This anticoagulant-preservative, citrate-phosphate-dextrose (CPD), was found to be superior to ACD in that 80 percent of the red cells retained viability after 32 days storage. Other studies (Refs. 21, 23, and 28) have confirmed that CPD stored erthrocytes have better survival than those stored in ACD, although not to the extent originally claimed. In addition, DPG levels in CPD-stored blood are better maintained; levels appear to be normal for approximately 1 week of routine blood bank storage (Ref. 35). Appropriate testing also has demonstrated that other blood constituents are not affected deleteriously by this anticoagulant when compared with ACD (Refs. 37 and 39). The formulas for ACD and CPD solution are contained in 21 CFR 640.4(d). In 1971, CPD was licensed as an anticoagulant-preservative by the Bureau of Biologics and has largely replaced ACD in general transfusion practice.

d. *Recommendations for Whole Blood (Human).* The Panel recommends that Whole Blood (Human) ACD and CPD be placed in Category I with changes in collection, processing, and labeling as discussed subsequently in this statement. The designation "Human" should be deleted from the product names.

2. *Possible hazards of red blood cell product transfusion*—a. *Metabolic problems related to transfusion*—(1) *Introduction.* Numerous metabolic changes occur in blood during storage. These are of clinical importance only when transfusion is massive, rapid infusion of large quantities of blood relative to the recipient's blood volume, or when the recipient is particularly susceptible to these changes. In all other cases, the metabolic complications resulting from transfusion are minimal. The important changes are discussed in the next 14 sections of this statement.

(2) *Changes in blood during storage in citrate.* Red blood cells stored in ACD or CPD undergo a progressive loss of ATP, loss of membrane lipid, increased rigidity, loss of 2,3-DPG which begins

immediately in ACD and after 1 week of storage in CPD, loss of intracellular potassium, and gain of intracellular sodium. After transfusion, the electrolyte changes reverse, ATP and 2,3-DPG are restored to normal, shape and flexibility return to normal, and the life span for surviving cells is unimpaired. The loss of membrane lipid and increased susceptibility to osmotic hemolysis are not corrected (Refs. 194 through 196).

The plasma of stored whole blood contains a number of potentially significant abnormalities: about 14.5 millimoles of citrate per liter which is in excess of the amount required to bind ionized calcium in the blood collected, a high lactate level and consequently a large metabolic acid load (25 to 40 mEq/L), potassium levels up to 30 mEq/L by the third week of storage, ammonia levels up to 1 mg/dL, a variety of vasoactive agents, and denatured proteins. If given rapidly without warming, blood carries a significant negative thermal load (Refs. 112 and 197).

(3) *Citrate toxicity.* Citrate avidly binds ionized calcium. This is why it is effective as an anticoagulant. To guarantee complete anticoagulation, there is a deliberate excess of citrate in each unit of blood. Therefore, recipients of massive transfusion are receiving not just blood that is free of ionized calcium, but also an infusion of free citrate. Theoretically, death can result from the depression of ionized calcium levels below a critical level in the recipient. This can be prevented by the administration of ionized calcium during the infusion of citrate. However, the quantitative aspects of calcium balance change rapidly in vivo, and a great excess of ionized calcium is as lethal as is a severe deficiency. Empirical treatment can thus be quite dangerous.

The patient's defenses against citrate infusion fall into two categories, the removal of the infused citrate and the mobilization of ionized calcium. Citrate is removed primarily by metabolic conversion. Since citrate is a normal intermediary metabolite and part of the tricarboxcylic acid cycle, it is methabolized in every nucleated cell in the body. While citrate levels in the recipient do rise during rapid transfusion, marked elevations are usually found only at transfusion rates of greater than one unit of blood every 5 minutes for the normalsized adult (Ref. 198 through 200). The citrate concentration in recipients falls rapidly upon slowing or stopping the transfusion.

Bunker et al. (Refs. 198) reported impaired rates of metabolism of citrate when the recipient was hypotensive, had diffuse liver disease, or was allowed to become cold. Howland et al. (Refs. 199) found more evidence of impairment with hypotension alone. As hypoperfusion, liver disease, and hypothermia can often be found in massively transfused patients, these critical points need more careful documentation. In massively transfused combat casualties in Vietnam, transfused lactate was found to be removed very rapidly, presumably indicating a similar efficiency in handling transfused citrate because of the similar mechanisms for the metabolic conversion of both organic acids (Ref. 201).

The ability of the transfused patient to mobilize calcium in his or her defense depends on two factors: the release of parathyroid hormone (PTH) and the mobilization of calcium from bone by the effect of the newly released parathormone. The rise in parathormone levels in response to the infusion of citrate is extremely rapid (Refs. 202 and 203). This implies release of preformed PTH and raises the question of whether sustained infusion of citrate, as occurs during extended massive transfusion, can be handled adequately once the stores of PTH have been depleted, and the response then relies on synthesis of new hormone. Blood flow to bone is significantly reduced in hemorrhagic hypotension. This must result in impaired mobilization of calcium, but studies on calcium mobilization following hemorrhage are lacking. Several studies (Refs. 204 and 205) indicate that the relationship between the blood citrate level and the ionized calcium level is not a set one, indicating that the patient's mobilization of calcium can be an effective response even when citrate cannot be removed completely.

These relationships are further complicated by the effect of blood pH. Alkalosis increases the binding of ionized calcium by plasma proteins and, at least in some animals, appears to produce a decrease in the concentration of total calcium in the blood (Refs. 206 through 208). Alkalosis also leads to higher blood levels of citrate and higher tissue levels, especially in the renal cortex and less so in the liver, implying impaired metabolism of citrate (Ref. 209). There have been no studies, however, on the effect of alkalosis on the ability to metabolize infused citrate. In the often-quoted study of Bunker (Ref. 198), clinical signs and symptoms of hypocalcemia were related blood levels of citrate, but estimations of the ionized calcium were made without regard to changes in pH. Sodium citrate was used as the anticoagulant in these studies and produced a marked alkalemia in the recipients, some of whom exhibited a decline in serum total calcium during the citrate infusions rather than the expected rise. Other investigators (Refs. 199 and 210) using standard anticoagulant infusions instead of sodium citrate have noted much less change in human volunteers at similar rates of anticoagulant infusion.

More recently, a number of clinical studies (Refs. 211 through 215), using the calcium ion sensitive electrode, have shown that there is some depression of ionized calcium level in most transfused patients, but the physiologic significance of this degree of depression remains obscure. When studied, the recovery was very rapid after transfusion ceased. An exception is the study of Cooper et al. (Ref. 216) in which rather significant changes in myocardial contractility were related to depression of the ionized calcium level in the blood of patients coming off cardiopulmonary bypass. It is possible, of course, that such patients may be unusually sensitive to alterations of ionized calcium levels as appears to be true for their sensitivity to alternations of acid base balance. In addition, the rates of transfusion were very rapid. Another, more recent study (Ref. 215), demonstrates no reduction in cardiac output despite lowering of the ionized calcium levels.

Objections to the routine administration of calcium after transfusion have been raised on the basis of sporadic case reports of severe iatrogenic hypercalcemia (Ref. 217). This potentially important problem is still handled in an empirical manner because of the lack of adequate data. The calcium ion sensitive electrode may allow some of these old questions about citrate toxicity to be answered adequately. Perhaps some readily acceptable and practical criteria, such as on-line analysis of electrocardiographic changes as indicators of the need for ionized calcium supplementation can be developed. For the time being, it is probably safe to assume that a normothermic adult with a functioning liver can tolerate a unit of blood every 5 minutes without requiring supplemental calcium. Empirical supplementation with ionic calcium is rarely necessary, but should be considered for those recipients who are doing poorly without apparent cause.

(4) *Coagulation abnormalities.* One complication of massive transfusion is pathological diffuse bleeding. This not only interferes with efforts to control the

original hemorrage, but in turn, leads to the requirement for more blood replacement. Such bleeding abnormalities are often attributed to the dilutional effect on coagulation factors of the transfusions themselves.

Some coagulation factors are absent or deficient in stored blood. Platelets, even if present, do not function normally. Depending upon storage time, concentrations of Factors V and VIII may be as low as 15 to 30 percent, and Factor XI may be 20 percent of normal. Ionized calcium of course is absent, but it is not likely that this has an anticoagulant effect because the cardiac effects of severe hypocalcemia are probably lethal before levels low enough to interfere with coagulation are reached.

Published studies on the coagulation changes in massively transfused patients show a variety of clotting abnormalities such that it is difficult to assign a pattern to the defects (Refs. 218 through 225).

Three groups of observations, however, indicate that the clotting abnormalities in massively transfused patients are due to more than simple dilution from transfusion with stored blood. These observations suggest a superimposed abnormality of clotting factor consumption, presumable disseminated intravascular coagulation (DIC). There is often depression of the various clotting components to levels lower than could be expected from the degree of exchange transfusion that has occurred. In addition, these patients often have hypofibrinogenemia, which cannot be explained by transfusion with stored blood since the fibrinogen content of stored blood is normal. Finally, in some seriously injured patients, marked abnormalities of the coagulation system existed before or very early during the course of transfusion. In these patients, the coagulation abnormalities tended to be corrected during the course of transfusion and reverted to the pretransfusion levels when transfusion was stopped (Ref. 221). Taken together these findings suggest that DIC is one cause of the major coagulation abnormalities found at times in massively transfused patients.

Important confirmation of this concept is found in data presented recently on massively transfused patients (Ref. 226). When a series of coagulation test results were plotted against the amount of blood transfused, there was little evident correlation, but when the patients were grouped according to the amount of time they remained hypotensive, there was a strong correlation. The more prolonged the hypotension, the worse the coagulation abnormalities at the same level of transfusion. This implies that perfusion was the critical variable, not the quantity of blood transfused.

The pathogenesis of DIC with massive transfusion is unclear. Hypoperfusion and extensive tissue damage or necrosis almost certainly contribute. Transfusion itself may also facilitate the development of DIC by a number of mechanisms. There is probably partial activation of some clotting factors during storage, and breakdown of platelets, white cells and some red cells which release thromboplastic material into the stored blood. A hemolytic transfusion reaction is a well-recognized cause of DIC. Whatever the cause for the DIC, the key to management appears to be maintenance of good perfusion for several hours during which the patient will often correct the continuing consumption of clotting factors. This, of course, can be difficult in a massively bleeding patient. There is little indication for heparin therapy in such patients, and enthusiasm for this therapy is waning.

In addition to DIC, impaired platelet function has been associated with massive transfusion. While dilutional thrombocytopenia has been noted repeatedly in these patients, the platelet counts are rarely below 50,000 per cubic millimeter, a level sufficient for adequate hemostasis in most circumstances. A recent study (Ref. 225) on heavily transfused trauma patients suggests an impairment in platelet function but it is not clear if these function tests were corrected for the lower platelet counts seen. DIC itself can cause impaired platelet function. This is an important question because an induced defect in platelet function would complicate management of the bleeding problem and increase the need for further transfusion, but is potentially correctable with platelet concentrates. Platelet function should be a focus of attention in current and subsequent studies on the problems of massive transfusion.

(5) *Acid-base alterations* (Ref. 201). Three-week-old stored citrated blood contains an acid load of up to 30 to 40 mEq per liter, and patients requiring massive transfusion often have a preexisting metabolic acidosis. Studies in animals indicate that the acidity of stored blood may contribute to failure of resuscitation from hemorrhage. It is understandable, therefore, that some authors have proposed the routine administration of alkalinizing solution during massive transfusion, supporting these suggestions with uncontrolled clinical observations.

The situation, however, is more complex than it appears. The excess acidity of stored blood is due mainly to the citric acid in the anticoagulant and the lactic acid generated during storage. Both are organic acids, normal intermediary metabolites, and are rapidly metabolized under normal conditions. The preexisting acidosis is also a lactic acidosis which can be reversed rapidly when perfusion is restored. The impact of the transfusion of stored blood on the acid-base status of the recipient then is not one of simple buffering in a closed system, but is rather complex involving rates of administration, metabolic removal, and the changing status of peripheral perfusion in the recipient which relates both to production and metabolism of lactate.

Studies in Vietnam showed that patients whose blood pressure was restored by the transfusion, and even some who remained hypotensive, easily handled the infused acid load of transfusion and reversed the preexisting metabolic acidosis (Refs. 201 and 227). Those in whom hemorrhage was not controlled during transfusion, most of whom died, exhibited increasing acidosis that was probably due more to persistent and worsening hypoperfusion than to the transfusion itself. Administration of alkalinizing agents to this latter group was futile. Besides being unnecessary, the routine use of alkalinizing solution presents several disadvantages. An induced alkalemia will impair oxygen delivery by shifting the oxyhemoglobin dissociation curve to the left, will increase the sodium load in patients whose ability to excrete salt and water is impaired, and may delay the return of ionized calcium to normal levels following cessation of the infusion.

The results discussed above were obtained in previously healthy young adults whose resuscitation was carried out in a high ambient temperature that prevented the development of hypothermia. In these patients failure to restore perfusion was almost always due to failure to control hemorrhage or to injury to a vital organ, rather than to acidosis per se. The need for alkalinizing agents should be based on objective measurements of blood gases and pH rather than on empirical formulas.

One clinical situation in which acidosis may occur during massive transfusion is exchange transfusion in the newborn. One recent study in infants indicated that transfusion-induced acidosis was not a problem (Ref. 228). The blood used, however,

was 1 to 4 days old and therefore had less lactic acid than is found in blood approaching the third week of storage. Also, none of the infants were less than 5 days old.

(6) *Alterations in oxygen transport.* In the simplest of terms, the oxygen delivering capacity can be thought of as a product of blood flow and oxygen content. Oxygen content, in turn, is a function of hemoglobin concentration and oxygen saturation. Recently, an additional aspect, the affinity of hemoglobin for oxygen, has been of interest. At physiologic gas tensions, a decreased affinity of hemoglobin for oxygen leads to less oxygen uptake in the lungs, but more oxygen release at the periphery, resulting in increased delivery of oxygen, independent of changes in flow or the concentration of hemoglobin (Refs. 229 and 230). A more complete description of the oxygen delivering system then includes blood flow, hemoglobin concentration, oxygen saturation, hemoglobin oxygen transport efficiency, and tissue oxygen tensions which reflect the metabolic need for oxygen. All five of these parameters can be altered by transfusion.

Blood flow can be increased by increasing blood volume in hypovolemic patients. Paradoxically, blood flow can be decreased by transfusing patients to the point of heart failure secondary to hypervolemia. The concentration of hemoglobin is increased by transfusion but the relationship to oxygen delivery is complicated by the fact that an increasing hematocrit is associated with increasing resistance of blood to flow due to increasing viscosity. This relationship is complex, with several variables poorly understood (Refs. 231 through 233). For the severely anemic patient, however, improved delivery of oxygen almost certainly results from transfusion. The saturation of hemoglobin with oxygen will decrease if transfusion leads to circulatory overload and consequent pulmonary edema. Restoring volume and/or hemoglobin concentration toward normal will increase the oxygen tension of mixed venous blood and indirectly will lead to higher arterial oxygen tensions even if the degree of "shunting" in the lung does not change. Finally, the efficiency of hemoglobin as an oxygen delivering agent is also altered by transfusion, and most of the remainder of this section will consider this relationship.

The concentration of 2,3-diphosphoglycerate acid (2,3-DPG) falls essentially to zero by the third week of storage of RBC in either ACD or CPD, although at different rates in the two solutions (Ref. 234). The 2,3-DPG depleted blood has an increased affinity for oxygen, making it a less efficient oxygen delivering agent at physiologic gas tensions. In most clinical situations of stress on the oxygen-delivering system, the affinity of hemoglobin for oxygen changes toward reduced affinity resulting in increased effectiveness of delivery. Patients massively transfused with old stored blood have an alteration in hemoglobin oxygen affinity opposite in direction to what would presumably occur in the absence of massive transfusion (Ref. 235). Several clinical studies have shown that a marked change in the affinity of hemoglobin for oxygen can result from massive transfusion (Refs. 236 through 243). Recovery of 2,3-DPG content and of normal oxygen transport function occur within approximately 1 to 2 days after transfusion of depleted red cells.

In spite of the obvious theoretical considerations, the detrimental effects of an increase in oxygen affinity in stored transfused cells have been difficult to demonstrate. Studies on massively transfused combat casualties in Vietnam (Ref. 201), who routinely received ACD blood in the second or third week of storage, indicated not only good resuscitative effectiveness, but immediate restoration of aerobic metabolism as indicated by rapid clearing of elevated pretransfusion blood lactate levels and intraresuscitative lactate loads.

Studies in rats (Ref. 244) and in humans (Ref. 245) have shown that lowering the 2,3-DPG content and increasing the affinity of hemoglobin for oxygen produces essentially no detectable effects on work performance or maximal oxygen consumption.

Almost all studies showing a detrimental effect of increased affinity of hemoglobin for oxygen have been complicated by the presence of anemia, while the studies showing a minimal physiologic effect were carried out at normal hemoglobin concentrations. The interaction of anemia and 2,3-DPG has been studied (Ref. 246) as related to the ability of rats to survive hemorrhage. At nearly normal hemoglobin concentrations, transfusion with red cells depleted in 2,3-DPG did not impair the ability of the animals to survive two different levels of hemorrhagic challenge. When the animals were studied at acutely anemic levels, however, transfusion with red cells depleted of 2,3-DPG was associated with a much higher mortality. These findings indicate that in stressful circumstances the resiliency and integrity of the oxygen delivering system is such that the degree of impairment produced by depleting red cells of 2,3-DPG is, by itself, insufficient to endanger survival, but that when combined with other circumstances such as acute reductions in red cell mass these shifts may become important.

There may be other circumstances where special attention must be paid to hemoglobin function. It is likely that a fixed and relatively low limit on cardiac output will also impair the patient's ability to compensate for impaired hemoglobin function so that these two together may assume new importance. Indirect evidence indicates that the newborn infant has a less well protected oxygen delivering system and is susceptible to conditions which would be tolerated by the adult.

Recently, Dennis et al. (Ref. 247) reported an increase in cardiac output when red cells rich in 2,3-DPG were used during cardiopulmonary bypass, using patients treated with red cells with lower concentrations of 2,3-DPG as controls. The differences in myocardial performance were greater than predicted from the small differences in hemoglobin oxygen affinity between the two groups, indicating that other variables must have been present. The same group (Ref. 278) subsequently reported better left ventricular performance in patients with abdominal aortic aneurysms operated upon electively and given relatively normal red cells compared with patients with ruptured abdominal aneurysms given old stored red cells. Obviously, hemorrhage was worse in the patient with ruptured aneurysm so it is difficult to see how any legitimate comparison can be made. Better controlled studies are needed on this important point.

(7) *Hypothermia.* Many studies (Refs. 248 and 249) have demonstrated that a massively transfused patient will become cold if the transfused blood is not warmed, especially if transfusion occurs in an air-conditioned operating room and is given to a paralyzed patient who may have one or more body cavities open to the air. It requires nearly 150 Kilocalories to warm five liters of blood from 4 to 37 °C. This is equivalent to the heat produced by 30 minutes of moderately heavy work and represents a requirement for an additional 30 liters of oxygen. This added requirement for oxygen for the production of heat may come at the time when the patient's survival is being threatened by a challenge to the oxygen delivering system.

It is widely assumed that hypothermia impairs the metabolism of citrate and of lactate, thus increasing the chances for developing hypocalcemia and metabolic

acidosis during transfusion. Cold increases the affinity of hemoglobin for oxygen, thereby worsening the impairment of hemoglobin function. Cold also promotes the release of potassium from the intracellular compartment which, when added to the increased chance for acidosis, might result in hyperkalemia that would not otherwise exist. Rapid transfusion through central venous catheters could differentially cool areas of the heart and may cause arrhythmias. Hypothermia, then, is potentially disastrous in the massively transfused patient, but many of these possible complications have not been documented.

At least two reported clinical experiences reported better patient survival during massive transfusion when the blood was warmed, but neither was adequately controlled. A recent study on exchange transfusion in the newborn could not document any detrimental effect from cold blood (Ref. 251), although the degree of hypothermia produced in adults in an operating room with an open abdomen is often much greater.

The potential for detrimental effect is apparent, and efforts should be made to warm the transfused blood in an exsanguinating patient. Unfortunately, blood can be hemolyzed by temperatures not far above normal body temperature; at the same time, rapid warming is needed so as not to delay the transfusion. All available devices exhibit some drawbacks (Ref. 252). The microwave equipment, in particular, has a small margin for error (Ref. 253). New devices are being constantly introduced and require careful evaluation.

(8) *Infusion of denatured proteins.* There is preliminary evidence that certain forms of storage may render blood, and particularly plasma, more harmful then beneficial to the acutely hemorrhaged animal. Controlled studies in rats (Ref. 254) indicate that plasma is inferior to almost any reasonable alternative as a treatment for hemorrhage. Denaturation is recognized as a serious problem in cardiopulmonary bypass. Even without cardiopulmonary bypass, foreign surface contact may alter the efficacy of shed blood when reinfused to treat hemorrhage (Refs. 255 and 256). Other studies reporting poor results when comparing the reinfusion of shed blood to other forms of treatment are characterized by harsh handling of the blood ex vivo. Both length of storage and specific plasma protein fractions have been found to make significant differences in the survival of animals treated for hemorrhage (Refs. 257 and 258). These findings indicate that biological effects can be related to the manner in which blood is handled during storage.

(9) *Infusion of vasoactive substances.* Much of the experimental work dealing with the release of vasoactive substances during storage has dealt with single donor or pooled plasma stored under conditions not necessarily applicable to the usual blood banking practices (Ref. 259). There has, in fact, been little study of the release of vasoactive substances into the plasma of stored whole blood. Serotonin levels increase as would be expected from the breakdown of stored platelets (Ref. 260). There is evidence that the kinin system is activated during storage, but much of this work was done on blood stored in glass rather than in plastic containers. It is likely that there is partial activation of Factor XII during storage, which in turn might lead to partial activation of the kininogen systems. The status of the prostaglandins, anglotensin and other as yet undefined vasoactive materials remains to be established. In the massive transfusion situation this array of vasoactive substances, many of which are associated with the production of increased capillary permeability and vasoconstriction, might explain some of the pulmonary problems occasionally encountered in massively transfused patients.

(10) *Impaired bacterial defenses.* There is suggestive evidence that massive transfusion with stored blood may impair antibacterial defenses (Ref. 261), probably through two mechanisms. First, blood that has been run through an extracorporeal circulatory device and then administered to animals produces an impairment of reticuloendothelial function (Ref. 262). More recently the same effects have been shown (Ref. 263) from simple storage of blood. The added observation has been made that less impairment of phagocytic function follows the transfusion of washed erythrocytes when compared with stored whole blood. The second possible mechanism relates to older observations (Ref. 264) that citrated whole blood decreases the phagocytic ability of polymorphonuclear leukocytes.

(11) *Hyperkalemia.* The potassium level in the plasma of stored whole blood rises to approximately 30 mEq/L by the end of three weeks, a 25 mEq/L excess over normal. Therefore, 10 units of blood may contain an excess of up to 75 mEq over the normal expected potassium content in plasma. This suggests that hyperkalemia might be a serious problem in massive transfusion, but the practical problem is less than it might seem. Most of the excess potassium represents leakage from intact viable red cells. Upon transfusion, these viable red cells reverse the lesion that occurred during storage and take up potassium (Ref. 195). Almost every study on massively transfused patients has shown hypokalemia rather than hyperkalemia, reflecting the metabolic and respiratory alkalosis which is so common in these patients (Refs. 222, 265, and 266). There is evidence, however, that even transient hyperkalemia may worsen the effects of hypocalcemia, in vivo as well as in vitro (Refs. 267 and 268). As this situation may occur at the peak rates of massive transfusion, it is possible that potassium may in fact cause trouble for patients in certain circumstances.

(12) *Impaired red cell deformability.* Concomitant with the fall in red cell ATP levels during storage, red cell membrane elasticity decreases so that stored red cells are less deformable than normal (Refs. 22 and 29). This has significance in terms of the function of transfused blood in the microcirculation because red cells have a greater diameter than many of the capillaries of the body which they must traverse and must deform to circulate. Nevertheless, the fact that such cells survive and function indicates that, by some mechanism or another, deformability is rapidly restored.

(13) *Elevated phosphate and ammonia levels.* Plasma phosphate and ammonia levels rise during storage. Even with CPD, however, the phosphate levels represent little threat in terms of the total body phosphate. Similarly, the ammonia in stored blood, as high as 1 mg/dL, is easily handled by a functioning liver and represents a problem only in patients with significant hepatic insufficiency.

(14) *Miscellaneous.* In the newborn, hypoglycemia has been reported as a consequence of exchange transfusion with CPD blood (Ref. 279), probably reflecting the release of insulin triggered by the high glucose levels of the stored blood.

3. *Plastic transfusion equipment—a. Introduction.* Compared with glass bottles, the advantages of plastic bags are: (1) Decrease in weight, (2) reduction in storage space, (3) reduction in breakage, (4) ability to prepare components in a sterile closed system, (5) ability to infuse blood under pressure without risk of air embolism, (6) elimination of risk of trauma to the recipient if the container should fall, and (7) easiness of handling.

Most plastic transfusion equipment consists of polyvinyl chloride (PVC),

plasticizers, a mold-release agent, and a variety of other compounds. Although it was recognized that constituents of PVC leach into the stored blood (Ref. 40), it was Jaeger and Rubin (Ref. 41), in 1972, who showed tissue deposition of phthalate ester plasticizers, specifically di-2-ethylhexyl phthalate (DEHP). This report led to renewed concern about the safety of plastic bags and the effects of the plastic, not only on red cells and other blood components, but on organ function as well.

b. *Factors affecting blood during storage in plastic bags.* (1) *Plasticizers.* Polyvinyl chloride, developed in 1933, is a rigid plastic, but the synthesis of DEHP in 1933 permitted interfusion of these two components to yield a plastic with flexibility, extensability, and workability (Ref. 42). Phthalate plasticizers are used extensively in commerce (Ref. 42). Approximately 1 billion pounds of 20 different phthalate compounds were manufactured in 1972 representing about 2 to 3 kg of esters produced for every citizen in the USA per year (Ref. 43). The food and medical products industry accounted for only about 5 percent of the total produced with the building and construction industry using 38 percent, home furnishings 19 percent, transportation 11 percent, and apparel 7 percent.

Diffusion of the plasticizer depends on its molecular weight, compatibility with the plastic, viscosity, affinity of the environment for the plasticizer, and temperature at which the plastic is used (Ref. 42). The plasticizer DEHP represents about 30 to 40 percent of the dry weight of plastic blood bags (Fenwal JA-2 blood pack) and is not firmly bound. DEHP is soluble in blood plasma, accumulating at about 0.25 to 0.361 mg/100 mL/day during whole blood storage at 4 °C with ACD or CPD solutions (Refs. 41, 44, and 45). There is no accumulation of DEHP in blood frozen at −80 °C or −150 °C for up to 2 years (Ref. 44).

There is essentially no DEHP in the anticoagulant during storage of the bag at room temperature (Refs. 44 and 46), and DEHP is not extracted by saline (Refs. 44 and 47). About 20 percent of the DEHP in stored blood is in the high-density (greater than 1.21) fraction of plasma, while 60 percent was found in the low-density lipoprotein fraction (less than 1.21) suggesting lipoproteins may solubilize DEHP (Ref. 47); washed red cells contain 4 percent to 6 percent of the DEHP found in the blood (Refs. 44 and 47). Compared with the progressive accumulation of DEHP in blood stored for 21 days, DEHP levels in plasma stored in plastic stabilize after 7 days with a peak of 120 μg/mL (Ref. 48). Even frozen plasma (−20 °C) accumulates levels of DEHP about half that of 4 °C stored plasma (Ref. 48). Among plasma derivatives, the fibrinogen fraction contains 18 to 39 μg/mL, albumin 5 to 25 μg/mL and immunoglobulins 63 to 160 μg/mL (Ref. 48). Of all blood components the highest accumulation of DEHP occurs in the supernatant plasma of platelet concentrates stored at 22 °C (Refs. 44 and 49). Levels of DEHP are 16.7 mg/100 mL after 48 hours of storage and 44 mg/100 mL at 72 hours. In contrast, stored platelet concentrates at 4 °C accumulate only 11 mg of DEHP per 100 mL at 72 hours.

DEHP is the only plasticizer known to leach from plastic blood bags, although other plasticizers are found in blood passed through commonly used biomedical tubing. Studies of tissue, blood, and urine levels of DEHP in man (Refs. 41, 45, 47 and 50) show no correlation between transfusion history and DEHP accumulation. DEHP can be isolated from tissues and urine of nontransfused persons suggesting the importance of other environmental sources of DEHP.

Storage studies with human blood in PVC bags compared to polyolefin from which no measurable DEHP is leached showed that microaggregate formation is not related to DEHP accumulation (Ref. 44). Also, DEHP could not be removed by the usual blood filters which remove microaggregates or by millipore filtration.

Neonates who had umbilical catheters used to administer fluids or blood and then died had higher levels of cardiac DEHP than control stillborns (Ref. 52). Higher levels were also found in the blood of infants dying of necrotizing enterocolitis. It is not clear whether DEHP accumulation is a cause of tissue damage or whether ischemic tissue accumulates DEHP more avidly.

One report has shown a decreased growth of human fibroblasts in tissue culture when the serum of the culture medium was obtained from 21 day old blood stored in plastic compared with blood stored in glass. Even more depression of growth was seen with serum obtained from the supernatant of 48-hour room temperature stored platelets (Ref. 53).

Other studies suggesting various toxic effects on drug metabolism and pulmonary, reticuloendothelial, and cardiac function have been done in animal systems using unphysiologic forms of DEHP, i.e., an emulsion or a poorly solubilized liquid solution (Refs. 51 and 54). Extensive studies by a manufacturer (Fenwal) in both animals and man using plasma extracted DEHP have shown no toxic effects (Ref. 45).

(2) *Bag thickness.* Viability of both red cells (Ref. 55) and platelets (Ref. 56) is influenced during storage by the ability of CO_2 and oxygen to pass through the plastic bag. Gas exchange is a function of bag wall thickness, agitation during storage, and storage surface area per unit volume of the blood product.

c. *Effects on cell viability and AHF activity.* Post-transfusion red cell survival varies among lots of plastic bags (Refs. 49 and 57). Whether this represents a result of plasticizers that leach during storage or some other variable is not known. Platelet viability is also related to the plastic bag (Refs. 58 and 60). Since viability appears to be better maintained at 22 °C than at 4 °C and the amount of DEHP leached is higher during 22 °C storage (Ref. 59), differences in platelet viability are unlikely to be due to plasticizers.

Studies by Kevy (Ref. 61) show a variable cryoprecipitate yield in vivo depending on the plastic bag used. However, no difference has been found in a subsequent report (Ref. 62).

d. *Summary.* The composition of the plastic bag used for blood storage influences the post-transfusion viability of red cells and platelets. Measureable quantities of the plasticizer DEHP accumulate during the blood storage but whether this is related to red blood cell viability derangements during storage is not known. Other potential toxic effects of DEHP on blood products or organ dysfunction following massive blood transfusion have not been well documented.

The production of blood bags and transfusion tubing needs to be standardized so that significant differences between lots do not occur. Furthermore, toxicology studies should be done only with plasma extracted DEHP rather than with other methods of administering the compound.

e. *Recommendations relating to plastic transfusion equipment.* (1) The evidence that leachables from plastic result in toxicity in vivo is not convincing and no action is recommended at this time.

(2) Research support should be provided for further investigation of possible toxicity of leachables in vivo, the interaction between plastics and blood components in vitro, and for the development of plastic bags and tubing which do not interact with blood.

(3) The production of blood bags and transfusion tubing needs to be standardized so that important differences do not occur between lots.

4. *Microaggregates*—a. *Introduction.* During storage of whole blood or red blood cells microaggregates consisting of platelets, granulocytes and possibly fibrin are formed. The number of microaggregates is proportional to the concentration of leukocytes and platelets in blood prior to storage and the progressive accumulation of aggregates is associated with decreasing levels of platelets and granulocytes (Ref. 63). The quantity of aggregates is higher in CPD than ACD blood during the first 24 hours but between 7 and 21 days the amount is the same (Ref. 63). Few aggregates form during the first 2 days of storage. The amount then increases slowly between 2 to 10 days (0.01 to 0.02 mg/mL) and then increases logarithmically during the remaining 21 days of storage (0.1 mg/mL) (Ref. 64). This rapid accumulation of aggregates after 10 days is associated with accumulation of fibrinogen in the aggregates (Ref. 64).

b. *Physiologic effect of aggregate infusion.* Microaggregates in stored blood have been suggested as a cause of pulmonary insufficiency following massive blood transfusion. To determine whether aggregates in stored blood cause or contribute to pulmonary insufficiency in the clinical setting has been a major problem, particularly since platelet microaggregates may be formed intravascularly in response to tissue injury (Ref. 65). Studies in dogs show that pulmonary damage associated with massive blood transfusion can be prevented by dacron wool filtration of stored blood (Ref. 66). Subsequent studies in the baboon also show pulmonary damage on histologic examination (Ref. 67), but this is not prevented by filtering the blood (Ref. 68). Whether these animal studies are applicable to man requires clarification.

One clinical study in man has suggested that pulmonary insufficiency can be reduced by blood filtration, but this was not adequately controlled (Ref. 69). A similar but more tightly controlled study in older patients transfused during elective operations on the abdominal aorta revealed little evidence of pulmonary dysfunction, and no difference between the group receiving blood through the standard filter and a similar group transfused through fine filters (Ref. 31). It has been suggested that pulmonary insufficiency may be more closely related to the type of patient injury than to blood transfusion. Studies on large numbers of transfused casualties in Vietnam yielded no convincing data suggesting that transfusion without special filtering produced pulmonary dysfunction (Ref. 32).

c. *Methods of aggregate removal*—(1) *Filtration.* Standard blood bank filters have a pore size of 170 microns and remove large debris. Filters available to remove microaggregates (16 to 170 microns) are made of: (a) Dacron wool, (b) polyester mesh (40 X 40 micron pore), (c) polyurethane foam, and (d) polyurethane foam-nylon wool. All remove particles greater than 40 microns, but filtration efficiency for smaller particles varies inversely with their resistance to gravity flow (Refs. 70 and 73), i.e., dacron wool, foam-nylon wool, foam and 40-micron mesh. The 40-micron mesh filter is efficient for volumes of 1,600 to 2,000 mL (Refs. 71 and 73), while the others have a limited efficiency for filtering more than 1 unit of blood (Refs. 72 and 73). Pressure filtration (150 to 200 mg Hg applied with an infusion cuff) removes particles with the same degree of efficiency with all filters except the 40 micron pore where pressure decreases the number of particles trapped (Refs. 71 and 73). Filtration of microaggregates is an efficient but costly method of removing microaggregates. Recent work using laser methods to measure microaggregates indicates that there may in fact be a paradoxical increase in the total number of particles with at least some of the fine filters used (Ref. 84). The advisability of using such filters might therefore be open to considerable question.

(2) *Other*—(a) *Age of blood.* Blood less than 2 days old has very few aggregates, and at less than 10 days old the quantity is still very small (Ref. 64).

(b) *Centrifugation.* (i) Centrifuging whole blood causes macroaggregates to form. These can be removed by a standard blood filter (Refs. 63 and 74).

(ii) Preparation of platelet and/or white-cell-poor before storage eliminates microaggregates (Ref. 74).

(c) *Washing with or without freezing.* Saline washing of red cells or freezing followed by deglycerolization and washing effectively removes aggregates (Ref. 74).

(d) *Agitation.* Continuous agitation during storage for 21 days decreases aggregate formation (Ref. 75).

(e) *Aspirin.* Addition of aspirin to blood prior to storage (Refs. 74 and 76) or ingestion of aspirin by donors (Ref. 76) is associated with lower quantities of aggregate formation.

d. *Summary.* Microaggregates progressively form during the storage of whole blood. The final volume of aggregates produced does not depend on the anticoagulant used (ACD vs. CPD). These aggregates might be partially removed by special filtration or by other means. Disadvantages in using microaggregate filters include reduced rate of infusion, added costs, and recent questions regarding overall efficacy. The value of removing these aggregates in preventing pulmonary insufficiency remains to be determined. In view of lack of evidence, there is no basis on which to recommend routine use of microaggregate filter at this time except in cardiopulmonary bypass where the filtering function of the lungs is absent.

e. *Recommendations regarding microaggregate filtration.* (1) Filters that remove microaggregates should be used in patients undergoing cardiopulmonary bypass.

(2) At this time there is insufficient evidence on which to recommend the use of microaggregate filters in other specific transfusion settings.

(3) Investigative efforts in this area should address the question of the optimal characteristics of a single filtering device to be used during transfusion and to be incorporated into the commonly used transfusion sets.

5. *Diseases transmitted by blood transfusion*—a. *Introduction.* A number of diseases, some of serious clinical importance, can be transmitted by transfusion. Since pathogenic organisms are found in different portions of whole blood, risks of post-transfusion diseases vary depending on the blood product or component used. In general, the risk for any disease is directly proportional to the volume of blood transfused.

b. *Viral hepatitis.* Since the first report in 1938 (Ref. 77), there has been increasing awareness of relationship between blood transfusion and some cases of viral hepatitis. Two distinct viruses have been identified as causing post-transfusion hepatitis (Refs. 78 and 79), and there is considerable evidence that additional, as yet unidentified agents exist (Refs. 80 and 81). Until recently, the inability to transmit the disease to most laboratory animals made research difficult and slow. There is now, however, a practical laboratory test which detects a surface antigen (HBsAg) of one causative agent, hepatitis virus B (HVB). The incidence of post-transfusion hepatitis has been estimated at 4.6 cases per 1,000 units of blood transfused, with one third of these being icteric (Ref. 82). More recent studies indicate a higher risk of between 7 to 10 percent of all blood recipients. The risk of post-transfusion hepatitis is increased when blood from commercial donors is used (Refs. 82, 83, and 85). Elimination of these donors along with routine testing of all donor blood by

sensitive tests for HBsAg, if performed by the most sensitive radioimmunoassay method, will reduce the occurrence of post-transfusion hepatitis. It is becoming apparent, however, that all cases may not be related to HBV (Refs. 80 and 81) or even the recently characterized hepatitis A virus (Ref. 80). Studies (Refs. 87 and 89) attempting to implicate cytomegalovirus (CMV) and Epstein-Barr virus (EBV) have been fruitless, suggesting that most cases of post-transfusion hepatitis result from infections with virus(es) not previously implicated.

Testing of large United States donor populations with sensitive third generation tests for HBsAg shows a carrier rate of 0.33 per hundred (Ref. 90). There is no evidence that recipients of blood containing anti-HBsAg have an increased risk of hepatitis B and no reason to test donors routinely for this antibody (Ref. 91).

c. *Transmission of other infectious diseases.* A syndrome of fever, lymphocytosis with atypical cells, negative heterophile test, inconstant splenomegaly, and mild hepatic dysfunction has been reported 2 to 6 weeks after transfusion (Refs. 92 and 94). Although originally labeled as the "post perfusion" or "post pump syndrome" it can occur after any transfusion of whole blood (Ref. 95). Studies have shown rises in anticytomegalovirus (CMV) titers in these patients supporting the hypothesis that CMV infections transmitted by blood transfusion may be the cause. One large study has shown serologic evidence of CMV infection in 35 percent of patients who had been infused with fresh whole blood (Ref. 96).

Heterophile-positive infectious mononucelosis, the clinical syndrome associated with the Epstein-Barr virus infection, has been transmitted by blood transfusion (Ref. 97). Anti-EBV responses have been reported in 8 percent of patients undergoing transfusions or extracorporeal circulation (Ref. 96). Both CMV and EBV appear to reside in leukocytes.

Transfusion-induced malaria in seen infrequently, although the incidence recently increased (Ref. 98). *P. malariae, P. vivax,* and *P. falciparum* all have been implicated, and a rise in the number of cases due to *P. falciparum* was noted coincident with the return of service personnel from Vietnam (Ref. 98). Current standards for donor acceptance, if followed rigidly, are adequate to eliminate nearly all potentially malaria infectious donors. The malarial parasites reside exclusively in erythrocytes and are transmitted only with transfusion of RBC-containing products.

Chaga's disease, African trypanosomiasis, kala-azar, toxoplasmosis, and infections with micro filaria can be transmitted by whole blood, but these complications are almost unknown in the United States.

Transfusion-induced syphilis, while it does occur, is not an important hazard today. This topic is discussed below regarding the serological test for syphilis.

6. *Hazards related to transfusion of blood elements other than RBC*—a. *Introduction.* Noneythrocytic blood elements—plasma, platelets, and leukocytes—are responsible for a variety of common transfusion problems. Although it would seem logical that red blood cells ("packed cells") which contain smaller quantities of these blood elements would have less risk than whole blood, there is no evidence to substantiate this notion. Problems related to plasma, granulocytes, or platelets are largely, but not completely, obviated by either simple washing or the washing associated with deglycerolization of frozen red cells. Lymphocyte-related problems may, however, still be present. Recent studies indicate that even after freeze-deglycerolization, there are significant numbers of viable lymphocytes in the final product (Ref. 102).

b. *Anaphylactoid reactions to IgA.* Severe transfusion reactions characterized by flushing, abdominal pain, diarrhea, chills, fever, edema bronchospasm, hypotension, and shock have been seen when IgA-deficient patients with antibodies to IgA are transfused with whole blood (Refs. 103 through 105 and 107). Although the incidence of absent IgA is about 0.1 percent (Ref. 106) and about 15 percent of these IgA-deficient persons have high titer antibodies to IgA (Ref. 108), anaphlactoid reactions from this cause are extremely infrequent.

c. *Pulmonary "hypersensitivity" reactions.* There have been several reports of normovolemic pulmonary edema after blood transfusion. In most cases they have been associated with leukocyte antibodies in donor or recipient (Refs. 108 and 109).

d. *Post-transfusion purpura.* A number of cases of acute, severe, thrombocytopenic purpura occurring approximately 1 week after transfusion of whole blood have been described (Refs. 110 and 111). In most cases the antibody in the recipient's plasma has been anti-PL^{A1}, and the patient's own platelets are PL^{A1}-negative. The donor platelets have been PL^{A1}-positive and incompatible with this recipient's antibody. The mechanism suggested in immune destruction of donor platelets by recipient antibody with concurrent immune complex-mediated autoimmune thrombocytopenia (Ref. 110).

e. *Graft vs. host reactions.* A disease process identical with the graft vs. host reaction has been reported in transfused children with immunologic deficiency (Ref. 113). Evidence obtained in vitro has shown that lymphocytes are viable in stored blood for 15 to 20 days (Ref. 115) and that patients who receive fresh or stored whole blood show evidence of cellular activation as manifested by a rise in atypical lymphocytes, or incorporation in vitro of ^{3}H-thymidine by autologous lymphocytes (Ref. 114). The relationship of these findings to the graft vs. host reaction is unclear, but is in keeping with the concept of lymphocyte stimulation by transfusion.

f. *Allergic reactions.* Allergic reactions, as manifested by pruritis or hives, are seen in about 1 percent of transfusions (Ref. 116). These reactions are usually mild and respond quickly to appropriate treatment, although occasionally an allergic reaction can be more serious. These reactions are presumably related to allergy to plasma factors.

g. *Reactions due to leukocyte antibodies.* The relationship between leukocyte antibodies and febrile transfusion reactions has been clear since 1957 (Refs. 117 and 118). The antibodies, formed as a result of alloimmunization during pregnancy or transfusion, react in vivo with donor leukocytes to produce a reaction marked by an initial flush, a latent period of about 60 minutes followed by a shaking chill, high fever, apathy, and generalized malaise. The development of these reactions in patients with appropriate antibodies is influenced mainly by the dose of white cells administered (Refs. 119 and 120). Fatalities have been reported.

7. *Other hazards of red blood cell product transfusion*—a. *Hemolytic transfusion reactions.* The reaction in vivo between blood group alloantibodies and red blood cells carrying the appropriate antigen is usually marked by an accelerated destruction of the red blood cells. The rate and site of this hemolysis and the severity of the clinical signs and symptoms accompanying it are dependent upon many variables, including class, titer, avidity, specificity, IgG subclass and complement-binding activity of the antibody, strength of the

blood group antigen, clinical condition of the patient, amount of blood administered, general anesthesia, and other drug therapy. Most hemolytic transfusion reactions result from clerical rather than laboratory errors. Misidentification of patients, blood samples, or blood containers is far more frequent and serious than the failure of appropriate laboratory tests to detect the incompatibility before the transfusion is administered. Cross-matching procedures, including the antiglobulin (Coombs) test, are required before transfusion in all but the most urgent cases and, if properly done, detect almost all potential incompatibilities. Prompt recognition and treatment of hemolytic transfusion reactions usually prevents the renal failure which is often seen after acute intravascular hemolysis. Disseminated intravascular coagulation has been reported in patients with hemolytic transfusion reactions (Refs. 121 through 123).

b. *Hemolytic transfusion reactions without demonstrable antibody.* Diminished survival of transfused red blood cells can occur without demonstrable antibody (Ref. 124). The hemolytic rate is generally moderate, although rapid hemolysis with clinical transfusion reactions has been reported. More sensitive methods for detection of antibodies in vitro may reveal some examples of antibodies that do not react by routine cross-matching procedures.

c. *Alloimmunization.* Blood transfusion is, of necessity, associated with the injection of many antigens into a recipient lacking of these antigens. Because of extreme complexity of the blood group systems, the HLA antigens of white blood cells and platelets, and the various polymorphisms of plasma proteins, it is impossible to give transfusions that are antigentically identical—or even similar—to the recipient. The red blood cell antigens A, B and RH_0 (D) are of sufficient immunizing potential that pretransfusion tests for them are routine so that these incompatibilities can be eliminated by donor selection. Transfusion to recipients who lack any of these antigens should be from donors who are also negative for the antigen in question. No such routine testing is required or recommended for other red blood cell antigens and, as a consequence of this practice, the risk of alloimmunization in recipients is 1 percent per unit of blood transfused (Ref. 125). Alloimmunized recipients are at increased risk when additional transfusions are required since the alloantibody may be directed against a high-frequency red blood cell antigen making it difficult or impossible to find compatible blood. In addition, the antibody may not be detectable at the time of pretransfusion testing so that apparently compatible blood will lead to an anamnestic response and a delayed hemolytic transfusion reaction. Such reactions are serious and, at times, fatal.

Alloimmunization to white cell and platelet antigens is directed predominately against antigens in the HLA system. HLA immunized recipients may have clinical reactions or, if candidates for granulocyte (Refs. 130 through 132) or platelet (Refs. 126 through 129) transfusions, may show decreased effectiveness of the transfusion.

Alloimmunization to plasma protein antigens is seen in the IgG, IgM, IgA, and lipoprotein systems. Anaphylactic transfusion reactions in such alloimmunized patients have been reported.

d. *Hemolysis from a mixture of blood with dextrose containing solutions* (Ref. 133). Transfusion reactions with intravascular hemolysis have occurred when whole blood was administered through a secondary recipient set containing solutions of dextrose alone. The mechanism of these reactions is related to osmotic swelling of red blood cells that are in contact with the glucose solution followed by lysis when the swollen cells are infused into normal recipients (Ref. 134).

e. *Bacteriologic contamination.* Transfusion reactions from bacterial contamination of blood are rare but serious occurrences. The high contamination rate (about 2 percent) (Ref. 135) seen in blood shortly after collection is not accompanied by clinical signs or symptoms at the time of transfusion because of the small number of bacteria present and their failure to survive or multiply during storage of the blood at refrigerator temperatures (Ref. 136). Those reactions that do occur are usually caused by gram-negative bacilli capable of growing in the cold (Ref. 137). Many of these organisms possess powerful endotoxins and, if transfused, bring about serious rections with a fatality rate of about 50 percent (Ref. 138). This problem has diminished dramatically with development of closed system processing and transfusion using plastic containers.

f. *Circulatory overload.* Circulatory overload is a common complication of whole blood transfusion especially when treating infants, patients with heart failure, and patients with chronic anemia. In the latter groups, cardiovascular compensation may be very precarious and the administration of unnecessary plasma in whole blood may precipitate left ventricular failure and pulmonary edema. Without monitoring pulmonary capillary wedge pressure, which is rarely indicated for the administration of blood, it may be impossible to detect early left ventricular decompensation before the onset of pulmonary edema. The resultant hypoxemia combined with the anemia and acute ventricular failure can be disastrous.

8. *Indications for transfusion of red blood cell products*—a. *Introduction.* Despite a large and long-standing experience, the indications for blood transfusion are not always easy to delineate. This is to be expected because of the complexity of transfusion products, wide variations in the homeostatic capabilities of patients, and the multiplicity of clinical situations that may be encountered. Thus, rules of thumb about when to transfuse, optimal hematocrit levels, and other such notions are unrealistic. The following discussion analyzes the recognized indications for blood transfusion, particularly in regard to the treatment of acute and chronic red cell deficits.

b. *Anemia.* The correction of a red cell deficit is probably the commonest reason for transfusing blood. In considering the indications for transfusing one must consider risk, benefit, and need. Risk is discussed elsewhere. As noted, it may be lower with some types of red cell preparations than with others.

The benefits of red cell transfusions are not as clear as they may seem. The red cell mass in any patient represents a dynamic equilibrium between production and loss. Any disorders of production or loss that can be corrected, should be. This will be more effective than the temporary benefit of transfusing red cells. If the hematocrit remains elevated after transfusion it is because the balance between production and loss has changed favorably, not because the transfused cells are somehow persisting indefinitely.

Given that transfusion brings about only a temporary increase in red cell mass accompanied by significant risk, there are situations where such temporary benefits outweigh the risks. Increasing the hematocrit increases oxygen availability by increasing the oxygen content of blood, while at the same time decreasing oxygen delivery by increasing the intrinsic resistance to blood flow. In order to predict the optimal hematocrit under varying conditions, detailed and accurate quantitative data on blood viscosity are

required. The few studies in vivo that have been attempted indicate that the resistance of blood to flow is probably an exponential function of hematocrit, but with different relative changes in different vascular beds (Refs. 271 and 272). Factors other than hematocrit are also demonstrably important (Refs. 273 through 275).

Most authors agree that under the conditions that apply in the normal state, there is maximal oxygen delivery at normal hematocrits (Refs. 231 through 233 and 276). As flow rate decreases, there may be an advantage in moderate hemodilution, but more studies of this problem are needed under conditions that more nearly approximate those in vivo.

The hemodynamic changes in acutely anemic patients and animals have been reviewed recently (Refs. 280 and 281). Perhaps the most fundamental observation is that when other variables are kept constant, increased cardiac output is a most important compensatory mechanism to maintain nearly normal oxygen delivery. There are probably elements of both increased left ventricular work and decreased viscosity in the compensatory increase in cardiac output. Such changes in cardiac output can only occur if blood volume is maintained near normal. Therefore, it is mandatory that hypovolemia be avoided in the acutely anemic patient. Indeed, loss of blood volume is more detrimental to oxygen delivery than is loss of blood oxygen-carrying capacity except at extreme levels of anemia. The primacy of volume over red cell mass is a well recognized principle of resuscitation from hemorrhage.

In studies in patients with chronic anemias it has been difficult to detect a deficit in work capacity with hemoglobin concentrations as low as 7 grams per dL (Refs. 282 through 291). Other investigators have found evidence of only moderately impaired function (Refs. 292 through 296). This correlates with the failure to detect much increase in cardiac output at rest with similar hemoglobin levels (Ref. 297). Unfortunately, many of these studies were carried out before the awareness of the liability and responses of oxyhemoglobin affinity in vivo to stresses on oxygen delivery. By changing levels of 2,3-diphosphoglyceric acid (2,3-DPG) in red cells, these shifts could compensate for losses of red cell mass by proportionally increasing the efficiency of hemoglobin as an oxygen-delivering agent at physiologic gas tensions. In view of the importance of the affinity of hemoglobin for oxygen in the oxygen-delivering system, it is surprising how little has been done to investigate the interaction between hemoglobin function and hemoglobin concentration in the response to stress. This question is discussed in the section on Oxygen Transport.

The heart occupies a unique position in the oxygen-delivering system apart from its central role as pump. With loss of volume and/or loss of red cell mass as well as with most other acute stresses to the oxygen delivering system, the heart works harder to increase total flow or to minimize any decrease in flow at lower filling pressures. The oxygen requirements of the heart increase, either absolutely or relatively, at a time when systemic oxygen delivery is decreasing. Oxygen delivery to the heart itself occurs largely during diastole, and is threatened in the bleeding patient by lower diastolic pressure as central aortic pressure declines and less time in diastole as heart rate increases. Most other organs can greatly increase oxygen extraction by working at a lower partial pressure of oxygen, but in man this important mechanism is almost completely unavailable to the heart, which works at all times near the minimum functioning PO_2. This unusual constellation of circumstances, increased oxygen requirements, a tendency to decreased blood supply from purely physical factors, and inability to extract more oxygen from available blood, place extraordinary importance on the ability of the coronary vessels to dilate in order for the heart to perform satisfactorily and the patient to survive during hemorrhage. Against this background, factors such as hemoglobin function and hematocrit could conceivably become survival-limiting variables in patients with coronary vessel disease. There are a number of studies relevant to these questions which are beyond the scope of the current discussion, but some of these studies suggest that the heart with limited coronary perfusion may be very sensitive to the oxygen-delivering properties of the blood perfusing it (Ref. 86).

With respect to oxygen delivery as an integrated system, the body has regulatory mechanisms controlling a number of important components: cardiac output, blood volume, hematocrit, and 2,3-DPG levels. Presumably other variables are manipulated to give high efficiency to the cardiac mechanism, that is, maximum peripheral oxygen delivery per unit of energy consumed in pumping.

For the clinician faced with the problem of what is best for the anemic patient, it is important to keep in mind the risks of transfusion and the transitory nature of the improvement that results from transfusion. Chronic anemia down to hemoglobin concentrations that are half normal is tolerated even by patients who must perform physical work. Even acute anemia can be well tolerated at similar hemoglobin levels if demands on the oxygen delivering system are minimal. On the other hand, factors such as coronary insufficiency, fever, hypovolemia, and likely further acute loss of blood, greatly increase the risks of anemia.

There are no simple guidelines or rules for establishing the need for transfusion to correct anemia. Need will vary with circumstances, and the decision to transfuse must be made on an individual basis considering both the current status and projected future course of the patient and the risks of all courses of action.

c. *Hemorrhage.* Hemorrhage is a clear and logical indication for transfusion when a significant percentage of the patient's blood volume has been lost. Limited hemorrhage can be treated with simple salt solutions or natural or artificial colloidal solutions (Refs. 298 through 300). The use of such solutions results in hemodilutional anemia, and the considerations discussed in the previous section on anemia apply. These solutions can be used as the sole replacement for limited hemorrhages, to keep the patient alive until blood is available, or in combination with blood to lessen the total amount needed.

The important consideration in the management of hemorrhage is that blood volume must be maintained. Most patients can tolerate acute loss of half their red cell mass, but acute untreated loss of half a blood volume is usually fatal. Therefore, volume replacement, not necessarily in the form of blood, must be begun as soon as possible.

Simple electrolyte solutions are effective, safe, and practical for initial resuscitation from hemorrhage. Experience in Vietnam (Refs. 301 and 302) and detailed studies in the United States (Refs. 297 and 298) indicate that blood volume can be restored by electrolyte solutions after hemorrhage. With electrolyte solutions alone, however, each volume of lost blood must be replaced by three volumes of solution. Disadvantages are over expansion of extracellular fluid requiring subsequent excretion by the patient, failure of the central venous pressure to reflect dangerous degrees of overloading loss of efficacy of the solution in terms of the fraction retained

in the vascular space as hemorrhage progresses, and lowering of plasma colloid osmotic pressure. Advantages are as follows: Administration of electrolyte solutions can be quickly and safely begun even outside the hospital; there are no adverse reactions except for overloading; the risk of post-hemorrhagic renal failure is reduced; losses through sequestration (third space losses) can be treated as they occur. Artificial colloids preserve plasma osmotic activity, but those now available in the United States, dextran and hydroxyethyl starch, may interfere with clotting (Ref. 184). The natural colloids, albumin or albumin plus other plasma protein preparations, do not interfere with clotting and carry little risk of hepatitis, but they are expensive and occasionally of limited availability.

Whatever is used initially, as the hemorrhage approaches half the blood volume, replacement with blood will be necessary. The more rapid the rate of bleeding and the more significant the associated diseases or disorders, the earlier blood must be used. Nonblood replacement of blood loss should be continued only if the anticipated loss is limited, the patient is doing well, and their are no associated conditions that make acute anemia less tolerable.

In severe situations in which exsanguination is imminent, it may be necessary to use uncrossmatched blood in order to save the patient's life. If possible, this will be type and Rh specific. The military experience with the use of uncrossmatched blood has been excellent (Ref. 185), but few of those recipients had been transfused or pregnant previously, and the blood was tested for high-titer anti-A and anti-B. In extensive bleeding the cause of hemorrhage must be identified and corrected as soon as possible. Evaluation of the clotting system early in the course of the hemorrhage occasionally yields evidence of a treatable clotting defect. Some patients with rapid exsanguination must be operated on to control bleeding before resuscitation is complete, as the maximum possible rate of administration of fluid and blood may not equal the rate of loss.

Monitoring central venous pressure can be helpful in the extensively hemorrhaging patient, but may not reflect overloading with simple salt solutions or, occasionally, acute left ventricular failure. Pulmonary capillary wedge pressure is more informative, but carries added risks and should be reserved for difficult situations. Blood pressure, pulse rate, respiratory rate, state of consciousness, urinary output, and skin temperature and color all can be valuable indicators of the adequacy of perfusion, but any of them alone can be misleading. Hematocrit, is a measure of red cell concentration and reflects changes in whole blood volume only over a longer range of time than is appropriate for resuscitation. Hematocrit, therefore, does not accurately reflect acute blood loss. Similarly, the central venous pressure does not always bear a linear relationship to blood volume. The rate of change with fluid administration is often more informative than the absolute level itself. Central venous pressure monitoring is most valuable as an aid to the early detection of fluid overload when administering blood or other fluids with plasma specific colloid activity.

d. *Clotting abnormalties associated with massive transfusion.* Component therapy guided by appropriate laboratory studies is the rational way to manage clotting abnormalities. The use of platelet concentrates, fresh frozen plasma, cryoprecipitate, and cryoprecipitate-poor plasma are indicated in the appropriate clinical setting. The use of clotting fractor concentrates, which are prepared from large plasma pools, are not indicated in the usual massive transfusion setting because of the high risk of hepatitis and other hazards.

The use of blood transfusions to correct coagulation abnormalities implies the use of fresh whole blood. This becomes rational only when multiple clotting deficiencies are to be treated simultaneously, appropriate components are not on hand, there is a concomitant need for red blood cells, and fresh blood is easily available. Such circumstances are most likely to occur in a small hospital when the need arises for platelet replacement in an actively bleeding patient. Even here, the use of fresh whole blood should be considered a temporary measure until platelet concentrates can be obtained. For replacement of plasma clotting factors, except factor VIII and factor V, blood 24 to 48 hours old is adequate.

e. *Exchange transfusion.* Exchange transfusion is an established therapeutic procedure for hemolytic disease of the newborn (Ref. 277). Special precautions are necessary for this particular version of massive transfusion as the metabolic defense of these sick newborn infants are not as efficient as in adults, especially if the infant is premature. The blood must be warmed, generally should be less than 5 days old, and adjunct therapy with calcium chloride and sodium bicarbonate may be necessary. The exchange rate must be carefully controlled, intake and output balanced, and the infant monitored closely. Mortality rates of 1 to 5 percent have been reported, although most deaths were probably due to underlying disease.

In performing exchange transfusion in the neonatal period, whole blood (CPD), heparinized whole blood, and frozen red cells reconstituted with fresh-frozen plasma are used, each having a number of real and theoretical advantages. It has been suggested, when using citrate-anti-coagulated blood, that half the plasma should be removed (Ref. 269). This will reduce the risk of citrate toxicity and leave the infant with a higher hematocrit at the end of the procedure.

Exchange transfusion has been extended to several other conditions, usually various forms of poisoning or intoxication. Recently, exchange transfusion has been used in the treatment of disseminated intravascular coagulation in infants, but there are no controlled studies. Exchange transfusion is being evaluated in the high-risk infants and in infants with respiratory distress syndrome (Ref. 270). Preliminary results are encouraging. A modification of exchange transfusion has been used for hepatic coma in adults. Total body washout is achieved by rapid exchange with cold asanguineous fluid followed by a short run on cardiopulmonary bypass to support the circulation, restore temperature, and restore red cell mass. The most notable successes have been in patients with Reye's syndrome and not in those with hepatitis A or B. Again, proof of benefit by controlled clinical trials is needed.

f. *Nutritional deficiencies.* Whole blood is occasionally given to provide a "general support," or to treat hypoproteinemia with some degree of concomitant anemia, or even to prepare a nutritionally depleted patient for some form of stressful treatment. All of these approaches and concepts represents serious misconceptions about the efficacy of blood transfusion, the dynamics of blood cell protein turnover in disease and malnutrition, the optimal management of the undernourished patient, and even more serious misconceptions about the risks of transfusion. General support and the correction of malnutrition are not legitimate indications for transfusion.

g. *Wound healing.* Wound healing is impaired by protein deficiency and hypoproteinemia, but is not impaired by anemia. Transfusion to promote wound dealing is not indicated, quite the

contrary, this is usually a florid example of poor medical practice.

h. *Extracorporeal circulation.* Some blood is necessary for cardiopulmonary bypass, but most such operations are now done using very little blood in the priming solution, allowing hemodilution during bypass and slowly replacing red cell mass after bypass. It is difficult to justify the persistent use of large amounts of blood in the priming solution in some institutions or to understand the marked interinstitutional discrepancies in utilization of blood for similar bypass procedures.

i. *Recommendations regarding red blood cell product indications.* (1) Red blood cell products should be administered only after a responsible physician has evaluated the potential benefit in light of the known risks and hazards.

(2) Red blood cell products are indicated to correct a red cell deficit, chronic or acute, when the deficit is severe enough to cause signs or symptoms resulting from oxygen deprivation.

(3) Red blood cell products are indicated to correct a red cell deficit in the absence of signs or symptoms when there is a reasonable expectation that serious signs or symptoms may soon occur if transfusion is not given.

(4) Whole Blood transfusion indications:

(a) Whole Blood is indicated for the replacement of actual or anticipated blood loss, e.g., during the course of major surgery, when the loss is massive, or, if the loss is moderate, when accompanied by signs and symptoms of oxygen deficiency or shock and when simultaneous restoration of red blood cells and volume is necessary.

(b) Extensive blood loss is also an indication for the use of Whole Blood when recognized single or multiple coagulation defects are present. The use of specific blood components is preferable, but in an emergency, or when these are unavailable and there is no reasonable alternative, fresh Whole Blood may be used. In general, this approach is not as efficacious as the judicious use of specific components.

(c) Whole Blood may be used when exchange transfusion is indicated in hemolytic disease of the newborn, and, perhaps, in selected cases of hepatic encephalopathy, acute massive hepatic failure, sickle cell crisis, post-transfusion purpura, drug intoxication, and respiratory distress syndrome in the newborn.

(d) Whole Blood is indicated to prime the pump-oxygenator for extracorporeal circulation in those special circumstances where crystalloid priming fluid alone cannot be used.

(5) Red Blood Cells (Human) (red cell concentrate, "packed cells") is the product of choice to increase the oxygen carrying capacity of blood in most situations requiring transfusion. It can be used in place of Whole Blood if administered in combination with specific components, crystalloid solutions, derivatives, or volume expanders as indicated by the patient's clinical condition.

9. *The donor*—a. *Donor standards*—(1) *Introduction.* A person's suitability to donate blood depends on two general considerations: The donation will not be injurious to the donor and the donated blood will not be unnecessarily hazardous to the recipient. Overly restrictive criteria for donor suitability are harmful because they unnecessarily reduce the number of eligible blood donors, thereby creating difficulty in maintaining adequate blood supplies. It is commonly asssumed that only 3 to 4 percent of the eligible population donates in any year; however, this figure is probably inaccurate. The correct figure is probably about 8 to 9 percent, and up to 25 percent of the eligible population have donated at least once within a 10-year period (Ref. 139).

(2) *The physician's role in donor selection.* The Code of Federal Regulations (21 CFR 640.3) requires that a physician take responsibility for developing criteria for suitability as a donor, although he or she may delegate the day-to-day selection of donors to trained responsible individuals. The Code of Federal Regulations further mandates that a procedure manual describing these criteria be available in the blood bank. A physician need not be at a collection site. The American National Red Cross (ANRC) requires that donor selection be delegated to a person "no less professionally qualified than a Registered Professional Nurse" and that a physician or emergency medical facilities must be "available" and "no longer than 15 minutes away by automobile" from the blood-collecting team. Since there are few published data regarding how often serious reactions occur and whether they require the immediate availability of a physician, it is difficult to analyze this problem completely. A study by the United States Army found that 6.7 percent of their donors had reactions on the phlebotomy bed and another 3.8 percent after leaving the bed but before leaving the donor area (Ref. 140). In an unpublished study (Ref. 141) of 65,182 consecutive blood donations during a 12-month period, donor reactions occurred in 3.9 percent. Reactions classified as moderate or severe occurred in only 0.25 percent and only eight donors (0.012 percent) required medical consultation away from the donor site. Moderate or severe reactions include all instances where the donor lost consciousness no matter how transiently. There were no fatalities and no morbidity beyond the day of donation.

(3) *Donor safety.* 21 CFR 640.3 requires only that the donor shall be in good health, have a "normal" temperature and blood pressure, a hemoglobin level of more than 12.5 gm per dL (no distinction is made between males and females) and be free of any acute respiratory disease. Donations of a unit of whole blood may occur no more frequently than every 8 weeks, although this requirement and the blood pressure constraints may be waived in an otherwise acceptable donor at the discretion of an examining physician. All other donor standards listed in the CFR are clearly for the recipient's benefit.

(a) *Criteria to prevent anemia in the donor.* It is well recognized that normal hemoglobin levels are higher in men than in women. Thus, although not required by the CFR, most blood donor centers use a higher minimal level for men, 13.5 gm per dL, than for women, 12.5 gm per dL. The copper sulfate method for estimating safe minimal hemoglobin levels is in wide use today and uses two solutions of differing specific gravities, one for men, the other for women.

It has been suggested (Ref. 145) that the specific gravities of the copper sulfate solutions be increased to avoid phlebotomy of iron-deficient donors. To counterbalance an increased deferral rate of normal donors with this approach, it was also recommended that finger stick microhematocrits be performed on all those who "fail" the copper sulfate test. Spurious results, both high and low, may be obtained with the copper sulfate technique (Ref. 142) owing to technical errors, donor-sampling variables such as use of venous blood, ear or finger prick (Ref. 145), or donor variables such as hyperproteinemia (Ref. 143). Also, quality control on the copper sulfate solutions may not be adequate (Ref. 154).

Of major significance is the site of blood sampling for hemoglobin determination. It has been shown that samples obtained by ear prick give less consistent and generally higher hemoglobin values than samples obtained by finger prick or from venous blood (Refs. 189 through 191). In one

study, the donor rejection rate was 7.96 percent by finger prick and only 1.94 percent by ear prick (Ref. 189).

In healthy individuals with adequate dietary intake, the daily iron requirement depends primarily on age and sex. Within the usual age for donating blood, the donor's sex is the primary variable. Men lose about 1 mg of iron daily and absorb the same amount from the average daily dietary intake of about 10 mg. Menstruating women lose about 2 mg of iron per day and, without dietary supplementation, may or may not increase absorption sufficiently to prevent iron deficiency. Childbearing adds a significant additional requirement for iron in that a net loss of 400 mg occurs during pregnancy. Once iron deficiency anemia occurs, it is generally recognized that increased absorption of normal dietary iron cannot adequately compensate for the deficiency and drug iron supplementation is necessary.

Phlebotomy of 8 percent of the total blood volume, removal of approximately a unit of blood from an average person, causes a 6 percent drop in the donor's hemoglobin level within 1 week. Hemoglobin levels return to normal after another 2 weeks (Ref. 144). However, each 450 mL of donated blood contains about 250 mg of iron or about 5 percent of the total body iron. Thus, a frequent blood donor in good health can become iron depleted in a few years if there is inadequately increased absorption of dietary iron. In donors, particularly women, who have compromised iron reserves, inadequate intake, or impaired absorption, the occurrence of iron deficiency is inevitable.

The prevalence of iron deficiency and/or iron deficiency anemia in the normal adult population has been the subject of a number of studies (Refs. 146 through 150). Broad generalizations are risky because of a number of variables among these studies, particularly those related to dietary iron intake and to criteria for establishing iron deficiency and iron deficiency anemia. A recent study (Ref. 148) of a northwest United States population, using 3 parameters for evaluating iron status, found iron deficiency in 20 percent of menstruating women and in 3 percent of men. The data also suggest that about 10 percent of all persons who might be blood donors are iron deficient, and the current tests for anemia in the donor population would detect fewer than half of them.

The iron status of blood donors has been studied extensively. Decreased serum iron and/or increased iron-binding capacity (Refs. 147 and 152) or diminished stores (Refs. 146, 147, 151, and 152) have usually been found. As early as 1944, anemia was found to correlate with the number of previous blood donations (Ref. 153) and reduced serum ferritin levels have been correlated with donation frequency as well (Ref. 187). The inescapable conclusion of all these considerations is that iron depletion and occasionally iron deficiency anemia will occur with measurable frequency in the blood donor population that donates blood regularly, particularly in women donors.

Iron depletion without anemia does not seem to be associated with any morbidity in man. Mild degrees of iron deficiency anemia are also generally well-tolerated. However, it seems desirable to prevent the occurence of iron deficiency aneima among blood donors so that they would not be subject to unnecessary medical evaluations and would have adequate compensatory capabilities if unexpected hemorrhage should occur.

There are a number of ways of dealing with this problem. One approach is to reduce the permissible frequency of blood donation, particularly among at-higher-risk women donors. A second approach is to recommend iron supplementation after blood donation as is currently being done by the British National Blood Transfusion Service. Even this approach will not be entirely effective in preventing iron depletion in very frequent donors (Ref. 152). A third approach may be to increase the acceptable level of hemoglobin for determining donor suitability.

The presumed benefits of reducing the frequency of blood donation must be weighed against the possibility that such a regulation would reduce the blood supply in this country. While there are undoubtedly a number of donors who donate regularly every 8 weeks, these may represent a small percentage (1.7 percent) of all blood donated (Ref. 188). Irrespective of this, the issue appears to be one of balancing societal interests in the blood supply against those of the individual donor. Similarly, the benefits of iron supplementation to donors must be weighed against the occurrence of gastrointestinal distress from the medications and the possibility of masking iron deficiency from a pathological cause and thereby delaying detection and early treatment of disease.

(b) *The donor must have a normal temperature.* This standard seems directed primarily at protecting the recipient from possible infection from blood obtained from a febrile infected donor. Fever is a clinical sign usually indicating an underlying pathological process and this standard might protect the donor as well. The delineation of normal temperature is more difficult, however. The mean normal oral temperature in a number of studies was 98.34 °F with a standard deviation of 0.47. It has been calculated that the normal range of temperature would then be between 97° to 100.4 °F. Furthermore, there is a diurnal variation in temperature which may be as much as 3 °F (Ref. 99). Fortunately, the peak temperature usually occurs between 8 and 11 p.m., while the nadir occurs at between 4 and 6 a.m., both times not usual for blood donations. These data apply to the resting state; in more usual circumstances other factors, such as degree of activity, play significant roles. Pathological conditions also affect the temperature level significantly, usually by producing an elevation. Conditions which cause low temperature levels, such as hypothyroidism, are distinctly uncommon. Both AABB and ANRC standards require only that the donor's temperature be below 99.6 °F. The rationale behind this limit is not known. Whether increasing the upper temperature limit would increase the donor pool significantly without compromising the quality of the product is unclear.

(c) *Weight.* Although not required in the Code of Federal Regulations, both AABB and ANRC standards require donors to weigh at least 110 pounds if they are to donate one unit (450 mL) of blood. Although apparently an arbitrary standard, it seems a reasonable protection against acute hypovolemic hypotension which might occur in a donor below the weight standards. For example, a 110-pound, five-foot tall woman has a blood volume of about 3 liters. Phlebotomy of one unit of blood amounts to an acute hemorrhage of about 15 percent of her total blood volume, probably the upper limit of acute hemorrhage that is not associated with frequent postural hypotension. While it is recognized that blood volume estimation based on weight alone is inaccurate, the minimum weight criterion seems effective and not unnecessarily exclusive.

(d) *Pulse rate.* Although not required in the Code of Federal Regulations, both the AABB and ANRC have pulse rate standards (AABB 50 to 100 per minute; ANRC 60 to 100 per minute). Bradycardia and tachycardia may occur in normal persons. Here again, while arbitrary and occasionally excluding healthy donors, these standards are reasonable to protect prospective donors who might have heart disease.

As a general practice, donors with irregularities of pulse are referred to

supervisory personnel for evaluation. Donors with sinus arrhythmia or atrial premature contractions are usually acceptable. Similarly, healthy donors with physiological bradycardia, such as can be seen with trained athletes, may be accepted.

(e) *Age limits.* Although age limits are arbitrary, upper limits are based upon well established correlates of the probability of cardiovascular disease as a function of increasing age. The present regulations provide for individual judgments to be made beyond the usually accepted age range for donations.

(4) *Recommendations regarding donor standards.* (a) Acceptable minimum hemoglobin level for males should be increased to 13.5 gm/dL.

(b) Donors may not donate more than four times per year and no more frequently than every 8 weeks. Procedures to bleed donors more frequently may be established with the concurrence of the Bureau of Biologics if adequate means are employed to protect donors from the development of iron deficiency.

(c) Hemoglobin levels must be determined in a manner that correlates with concentration in venous blood.

(d) Research should be encouraged into the feasibility, acceptability and efficacy of administering iron supplements to blood donors.

10. *Testing of donor blood*—a. *Blood typing.* Characterization of the ABO and Rh types of all donor bloods is universally practiced and mandated in the Code of Federal Regulations (21 CFR 640.5). Although there are hundreds of blood group antigens on the red cell surface, those in the ABO and Rh systems are critical because of their immunogenicity, frequency distribution, and the dire consequences of transfusion when incompatibilities exist. The reliability of ABO and Rh typing depends largely on (1) the quality of reagents used for testing, (2) the tests that are performed, (3) the controls used in tests, (4) the competence of the technologist, and (5) the recordkeeping and organizational and operational abilities of the blood collection center.

b. *ABO typing.* The most important test is determination of the ABO type. The serious complication of transfusing ABO incompatible blood, frequently fatal when it occurs, demands that this test be performed by a dedicated technologist using the utmost care, highest quality reagents, and well-controlled labeling procedures.

ABO typing (also called grouping) is usually done with monospecific anti-A and anti-B reagents. In many laboratories, an additional test using anti-A, B (group O serum) is also done to check on the monospecific tests and to detect variants of A or B red cells. Most laboratories also perform so-called reverse grouping (serum typing or "back typing") in which the donor's plasma is tested with known type A and B RBC. This confirms the cell typing since normal persons have serum antibody specificity antithetical to the cell type.

Current standards for antisera used in ABO typing are the subject of another review in this document. These reagents are designed to give strong reactions when tested against cells specific for the labeled antigentic type and are more than adequate to facilitate accurate serological determination by even relatively unskilled technologists. There are, however, situations in which routine testing with anti-A or anti-B is not entirely adequate in that erroneous results will be obtained unless additional tests and/or control observations are made. These include:

(1) *Weak sub-groups of A or B antigens.* Numerous variants of the A antigen give weak or no agglutination when tested with usual blood-typing reagents. In some persons of group AB, the B antigen weakens the reactivity of the associated A leading to weakened agglutination when testing is done with anti-A reagents.

(2) *Positive direct antiglobulin tests.* Blood donors who have positive direct antiglobulin tests may be erroneously type as positive for certain red cell antigens as a result of excessive globulins bound to the red cell surface. This is true particularly with blood-typing reagents diluted in high-protein media or automated blood-typing systems.

(3) *Other conditions.* There are a variety of other conditions that may affect the accuracy of blood-typing reactions. These include acquired B antigens, hypoglobulinemia, hyperglobulinemia, polyagglutinable red cells, and recent previous transfusion with blood of a different type.

c. *Recommendations regarding ABO typing.* According to the Code of Federal Regulations (21 CFR 640.5(b)), blood shall be classified as to ABO blood group by at least two separate blood group tests, and blood-grouping results obtained by the different methods and with different lots of reagents must be in agreement. The Code is not specific about the tests required. In the interest of accurate ABO blood type determinations, the Code of Federal Regulations should be modified to be more specific as follows:

(1) Licensed anti-A and anti-B reagents or equivalent must both be used for determining blood type.

(2) Reverse grouping of donor plasma or serum against red blood cells of known ABO type (A and B) must be part of the routine testing.

(3) Cell and serum grouping must be in agreement before a final label is placed on the blood.

d. *Rh typing.* Typing for the Rh_o(D) antigen is routine on all donor bloods. Because the majority of Rh antibodies are of the coating ("incomplete") type, Rh typing usually is done in the presence of added protein and/or other diluent materials and a control test is required using a high protein solution and/or other diluent solutions without any anti-Rh_o(D) antibody. A positive control test suggests that the Rh typing may be erroneous.

If the test with Rh antisera is positive, the donor is considered to be "Rh positive." If there is no agglutination the donor may ge Rh negative, although some of the persons whose cells fail to react are, in truth, Rh_o positive. These persons are of the D^u phenotype and can be detected by incubating donor erythrocytes with Rh antibody, washing them, and then performing an indirect antiglobulin test. Only persons negative by direct typing and D^u testing are truly Rh_o negative.

Some laboratories further test Rh_o negative blood for other Rh antigens, particularly C and E. Approximately 97 percent of Rh_o(D) negative individuals have the Rh genotype *rr* (*cde/cde*) and thus also lack C and E antigens. In some laboratories the 5 percent of Rh_o(D) negative individuals who are positive for either the C or E antigen are generally not used as donors for Rh_o(D) negative (*rr*) recipients because of the possible immunization of the recipient to the C or E antigens. This practice is unnecessary and inconsistent because: (1) C and E are relatively weakly immunogenic, and (2) there are situations of Rh positive donor-recipient pairs where the donor has C or E while the recipient is negative for these antigens.

Rh typing is second in importance only to ABO typing. The Rh_o(D) antigen is probably the most immunogenic red cell antigen outside the ABO system. Thus, the likelihood of immunization when an Rh_o(D) negative recipient is transfused with Rh_o(D) positive blood is high, although by no means certain. Development of antibodies to the Rh_o(D) antigen compromises the safety of future transfusions should Rh_o(D) blood be used. It also has significance in women recipients with a change of childbearing because of the possible induction of maternal Rh_o antibodies that are crucial in the pathogenesis of hemolytic disease

of the newborn. These considerations make the requirement for Rh_o (D) typing of considerable importance.

The necessity of D^u typing has been questioned. If D^u testing were not done, these units would be labeled as Rh_o negative and transfused into Rh_o negative recipients. In one study of 45 donor recipient pairs (Ref. 155), there were no instances of immunization of Rh_o(D) negative recipients to the Rh_o(D) antigen after transfusion with D^u positive blood. Immunization with D^u blood has been reported rarely (Refs. 156 and 157), but in one case the specificity of the resulting antibody is in question (Ref. 158). Thus, the immunogenicity of the D^u antigen is low. However, D^u testing does afford an extra measure of protection, perhaps only theoretical, against immunizing women with a potential for childbearing.

e. *Recommendations regarding Rh typing.* (1) Testing for the Rh_o variant D^u is required. Blood that is D^u positive should be labeled Rh_o(D) positive.

(2) Routine testing for C and E or other antigens should not be required.

f. *Antibody detection tests.* Although not required by the Code of Federal Regulations, antibody detection testing on donor serum is common in most blood collection facilities. If the test is negative, the blood is labeled "no unexpected antibodies detected when tested by * * *." Unexpected antibodies are found exclusive of the normally occurring anti-A or anti-B alloantibodies. Unexpected antibodies found may or may not be identified, depending on the practice of the particular blood collection facility. The label must indicate that antibody is present, and if identified, the specificity must be noted. Such units may be transfused at the discretion of the transfusion service director and attending physician. The incidence of significant antibodies is low in healthy individuals who have never been pregnant or transfused with red blood cell products. It has been found (Ref. 192) that 0.322 percent of all donor bloods contain unexpected antibodies, 80 percent of these in women. Antibody screening eliminates the need for minor cross-matching prior to transfusion.

Considerable controversy exists over many facets of antibody screening. Should antibody screening be mandatory for all units of blood collected? If so, which test(s) is (are) most suitable? Should antibody identification be mandatory for those units in which antibody is detected. Which antibodies are clinically significant when transfused into a recipient? The question of clinical significance must be addressed in relation to the transfusion in prospect. For example, the presence of anti-Rh_o (D) in Rh negative donor blood may be significant if transfused into an Rh_o(D) positive recipient, but is insignificant if the recipient is Rh_o(D) negative.

It is advisable to do whatever antibody testing is required as part of the routine processing of donor blood. This permits detection of antibody-positive units at the time of collection rather than in the transfusion service where unexpected findings can delay transfusion to a patient. Serum from donors without antibody need not be tested against recipient cells (minor cross-match) eliminating that time consuming and, at times, confusing procedure in the pretransfusion evaluation.

Antibody screening at the collection center also leads to detection of rare donor units because the presence of antibody implies that the donor is negative for the corresponding antigen. These rare donors should be registered for future recruitment should there be a need for their blood. In addition, the red blood cells may be frozen for use when rare blood is required.

There are numerous procedures, both manual and automated, for antibody detection testing. The goal is to detect all clinically significant antibodies, but not necessarily all antibodies. While the question of which antibodies are clinically significant is thorny, there appears to be agreement that antibodies reacting only in vitrol at 5° C, and/or room temperature, but not at 37° C, usually are of little clinical importance. Most clinically significant antibodies belong to the IgG immunoglobulin class and a test to detect these is necessary. Within these constraints, there are numerous acceptable procedures for antibody screening.

Determination of specificity on antibody detected in donor plasma is desirable to identify special donors who might be needed in the future and to allow development of a frozen red cell inventory. Antibody identification could be done at the blood collection facility or the transfusion service, whichever has greater competence.

An alternative to testing all donor bloods for unexpected antibodies has been developed, which appears efficacious in ascertaining those units which contain antibody (Ref. 192). With this approach, only donors giving a history of previous pregnancy or blood transfusion and all Rh negative donors are tested for irregular antibodies. The incidence of undetected sensitized donors with this approach was estimated at 1 in 10,000.

While it is generally conceded that transfusion of antibody (outside of anti-A, anti-B, and anti-D) in donor plasma is of little clinical significance and there has probably never been a well-documented case of this hazard (Refs. 192 and 193), there appears to be no good way of ensuring this in every case. Undoubtedly, in some cases the passive transfusion of antibody results in a positive direct antigobulin test in the recipient and may create unnecessary laboratory confusion and testing if post-transfusion samples are evaluated. Furthermore, the amount of antibody transfused must be considered relative to the recipient's blood volume. Also, the amount varies with the blood product; whole blood contains about three times as much plasma as red blood cells, while deglycerolized red blood cells contain almost none. Problems with infusion of antibody are thus more likely to be seen with whole blood transfusion. The major drawback in labeling units of blood containing antibody is that some physicians will not use this blood in appropriate and safe settings because they do not understand the issues involved. This problem in medical education does not seem amenable to correction by compromising the quality of testing or by failing to disclose what the blood contains.

In summary, antibody detection and identification procedures on donor blood are valuable: (1) In identifying blood that might be harmful if transfused, (2) in identifying blood that might confuse future serological evaluation of the recipient, (3) in selecting donors who might be called when specific phenotypes are needed, (4) in permitting frozen storage of blood of known phenotypes, and (5) in identifying donors who could be used as a source of blood-typing reagents. Proper selection of test conditions and reagents is vital for adequate antibody screening and idenfitication. Routine testing is not generally useful at a temperature less than 20 °C, and conditions should be appropriate for detecting IgG coating antibodies. While the clinical importance of passive antibody transfusion is slight and is related to the product transfused, the size of the recipient, the specificity and immunoglobulin class and subclass of the antibody, and the presence of the corresponding antigen in the recipient, the designation on the label of any antibody specificity present permits the transfusion therapist and clinician to make sound clinical decisions with all the available facts. For frozen deglycerolized red blood cells, however,

such a designation is unnecessary, inaccurate and, perhaps, confusing.

g. *Recommendation regarding antibody detection tests.* (1) In general, each unit of donor blood should be screened for unexpected antibodies either using Reagent Red Cells intended for this purpose or by an equally effective method. Thus, in carefully selected populations an adequate medical history may be utilized in lieu of laboratory tests for unexpected antibodies provided the procedure meets FDA requirements.

(2) Donor blood found to contain significant antibodies should not be used for transfusion purposes unless the plasma has been removed.

(3) Tests utilizing donor serum and recipient cells should not be required as part of pretransfusion testing.

(4) If the unit of blood is to be transfused without significant levels of donor plasma, antibody screening and identification are not necessary.

(5) Research should be encouraged to develop alternative methods to detect and define antibodies that are of clinical significance.

11. *Testing for serological incompatibility (cross-matching).* The test for serological incompatibility (cross-matching) occupies a central role in determining safety of blood transfusions. It is the final laboratory test performed prior to transfusion and is an attempt to ascertain whether the donor red blood cells to be transfused will have a normal life span in the recipient. This procedure must be performed prior to transfusion except in situations where the time required for such tests would compromise the recipient's chances for survival.

Cross-matching is necessary for two primary reasons. The human red cell contains numerous antigens, each potentially immunogenic. It is impossible and undesirable to phenotype all potential blood donors and recipients in an attempt to transfuse completely matched blood; it is routine to type only for the A, B, and the Rh°(D) antigen. This practice is based on both the known disastrous consequences of transfusion of ABO incompatible blood and on the immunogenicity and relative frequency of the Rh antigen within the population. Furthermore, most transfusion services now routinely test potential recipient's plasma for unexpected antibodies by using specially prepared test cells which bear a large number of erythrocyte antigens. Antibodies in the recipient directed against antigens on donor RBC but not on the test cells are detected by the cross-match. Thus, the cross-match tests recipient's plasma for antibodies against donor RBC antigens (other than ABO and Rh) which may have been missed by routine plasma antibody screening procedures. A second, and no less important, role of the compatibility test is that it helps in discovering errors in ABO and Rh typing or labeling.

a. *Limitations of cross-matching.* Despite its importance, cross-matching has certain limitation:

(1) Not all errors in labeling or typing will be detected by this test.

(2) In vitro testing for serological incompatibility is not always predictive of survival in vivo. Recipient antibody against donor red cells does not always mean that, if transfused, the cells will have a shortened survival. Conversely, the absence of antibody as determined by the cross-match cannot ensure in vivo red cell survival.

(3) Cross-matching will not prevent the immunization of a recipient since it only detects performed antibody.

b. *Technical considerations regarding cross-matching.* The major cross-match tests donor erythrocytes against recipient serum and the minor cross-match tests recipient erythrocytes against donor serum. The importance of the major cross-match has never been questioned seriously because it is designed to detect the presence of antibody in the entire plasma volume of the recipient which is directed against donor erythrocytes. Whether antibody screening alone of recipients would suffice in certain transfusion settings is the subject of considerable recent discussion but beyond the scope of the Panel's work.

The minor cross-match is designed to detect a finite and relatively small quantity of antibody in donor plasma directed against the entire erythrocyte mass of the recipient. This latter test, widely used in the past, is unnecessary because:

(1) The majority of donor processing laboratories now do antibody screening on all donor bloods.

(2) Infusion of plasma containing antibody in the course of a transfusion results in an immediate dilution of that antibody approximately tenfold in the average adult, thus rendering the antibody harmless for the recipient in most cases.

(3) Unexpected donor antibodies almost never cause hemolytic reactions. For more discussion of this issue see section on Antibody Screening.

c. *Recommendations regarding cross-matching.* Tests for incompatibility shall employ methods that demonstrate significant hemolyzing, agglutinating, and coating antibodies which are active at 37° C and shall include antiglobulin tests or their equivalent.

12. *Serological testing for syphilis in blood donors.* The routine testing of blood donors for evidence of syphilitic infection became common practice in the era when blood was transfused in the fresh state and when there was only questionably effective treatment for syphilis. The transmission of syphilis by blood transfusion was a documented hazard with potentially severe consequences for the recipient. Testing of the donors at the time of collection seemed to be a logical and possibly effective control measure (Ref. 159).

Transfusion-related syphilis has not been reported in the United States for over 20 years. There is reason to believe that this success is not due solely or even primarily to routine serological testing of the donor. This practice, which has now become codified (21 CFR 640.5(a)), should be reexamined.

There are three hypothetical reasons for performing serological tests for syphilis (STS) in all blood donors: Prevention of syphilis in the recipient, detection of syphilis in an asymptomatic donor, and detection of "biological false positive" (BFP) reactors and removal of this blood from the blood banking system.

Routine STS probably has little to do with prevention of syphilis in recipients because the STS is often negative during the early period of infectivity. Further, most blood is now usually transfused after at least 4 days of liquid storage (exact figures are not available), which alone is sufficient to eliminate infectivity for syphilis (Refs. 100 and 160 through 163). It is quite likely that the great majority of the recipients of blood less than 4 days in storage receive antibiotic treatment because of the nature of their illnesses. Syphilis is no longer the dreaded, incurable disease it once was. If the control of syphilis were worth of being singled out for such attention, it would be more efficient to test the recipients 3 weeks to 4 months after transfusion. After 3 weeks, passively transfused regain will probably have fallen below the level detected by currently used STS, and any new syphilitic infection in the recipient would be manifested by a positive STS by 4 months (Ref. 164).

As a case-detecting and venereal disease-controlling strategy, routine STS of all blood donors has some rational basis, but the cost-effectiveness ratio should be carefully examined. In testimony in open session before the Panel, it was estimated that there is one true positive STS per 1,400 donor tests in the United States as a whole. Of 26,128 cases of primary and secondary syphilis reported to the United States Public

Health Service (USPHS) in 1975, only 154 were detected by screening blood donors (0.6 percent). Only 521 cases of syphilis in all stages were detected and reported, from an estimated 8 to 9 million tests. Even with this yield, more rigorous systems of followup on reported reactors and their contacts would be necessary in order for the program to be truly effective. If the act of blood donation is to be used as an opportunity to engage in the random screening of the health of asymptomatic adults, it is not logical to single out syphilis as the disease worth this effort.

The final consideration in STS of blood donors is the Biological False Positive (BFP) reaction. Recent data (Refs. 165 and 166) indicate that the BFP now accounts for 90 percent of the positive reactions to STS in the United States. If ture, this seems to reflect a continuing increase in this fraction, which was reported in the early 1950's as 50 percent in some areas of the United States (Ref. 167). The absolute percent of the total population with BFP (0.47 percent then versus 0.4 percent now) indicates that most of this trend is due to the reduced number of true positive reactors reflecting infection. It is clear that most positive reactors now have BFP reactions.

The BFP reaction seems usefully divided into two categories: acute (reversion to negative within 6 months in the absence of treatment for syphilis) and chronic (continued positive reactions in the continued absence of evidence of syphilis with more specific tests and on physical examination). The acute BFP reactions are associated with a wide variety of conditions, most of them infectious (Ref. 175). Most texts now state that almost any acute febrile disorder or immunization can result in an acute BFP (Ref. 168). Some of the better documented causes, however, are infectious diseases that could be transmited by transfusion where they could induce significant illness in the recipient, e.g., malaria, infectious hapatitis, infectious mononucleosis. The temporal relationship between infectivity as a blood donor and the development of a BFP is not known for these illnesses. Other causes for BFP mentioned are Hodgkin's disease, sarcoidosis and, increasingly, intravenous drug abuse. There is some dispute about the role of repeated blood donation in causing BFP reactions.

The chronic BFP reactors are an interesting group of patients who have been studied in detail and followed carefully in several investigations (Refs. 167 through 169). Up to 80 percent develop manifestations of dysgammaglobulinemia. As many as one-half develop laboratory and clinical signs of a "collagen disease," usually systemic lupus erythematosus (SLE), within 10 years. Only about one quarter remain seemingly healthy 10 years after their detection as a BFP reactor. The disorders associated with BFP reactions are serious, sometimes fatal, diseases without known cause or cure. There is no evidence that these conditions can be transmitted by blood transfusion. Conversely, their very slow onset and gradual development make it unlikely that transmission by transfusion would be readily detected. In fact, it would require very long range, careful followup of a type that is rarely done to detect this result because the latent period can exceed 10 years in the donor.

a. *Summary regarding the STS.* The mandatory STS on all blood donors represents an unusual regulatory situation. The practice was instituted for good reasons and was initially probably beneficial to donors and recipients. Both the potential infectivity of the blood and the treatment of the disease have changed drastically, however, and the STS probably has little value now in preventing the transmission of syphilis by transfusion. It may have some small value in case-detection of syphilis, but this does not seem an appropriate indication to mandate its continued use. There is no evidence that the biological false-positive STS reaction is associated with any known acute or chronic diseases that might be transmissible by transfusion. Nevertheless, because the frequency of the BFP reaction is relatively high, it might indicate the presence of a transmissible disease.

b. *Recommendations regarding the STS.* The STS should be eliminated as a mandated test on donor blood prior to release for transfusion.

13. *Testing for hepatitis.* Estimates of the incidence of post-transfusion hepatitis are difficult to find. In 1970 (Ref. 172), the National Research Council (NRC) estimated that perhaps there were 30,000 cases of clinical hepatitis annually, with a mortality rate of 10 percent. They also suggested that there may be up to five times that many cases when one considers subclinical cases. More recent studies (Ref. 173) estimate the risk of post-transfusion hepatitis at 6.4 cases per 1,000 units of blood transfused; one-third of these are icteric. This estimate is consistent with the earlier one of the NRC. The difficulty in defining the precise risk is related to numerous variables including ineffective reporting and followup, variable criteria for diagnosis, and source of donor blood from both a geographic (Ref. 174) and socioeconomic (Refs. 82, 176 and 177) point of view.

Two risk factors for post-transfusion hepatitis have been clearly identified. Blood obtained from paid blood donors carries a higher risk (Refs. 82, 176 and 177), although payment per se does not make the blood more dangerous. Undoubtedly, socioeconomic factors related to donor recruitment are important. The second risk factor is the presence of the hepatitis B surface antigen (HBsAg) (Refs. 178 through 182). The introduction of universal HBsAg testing on all blood donated in the United States, particularly with sensitive third generation tests, has been associated with a reduced incidence of post-transfusion hepatitis. Eliminating the commercial blood source would be even more effective in this regard (Ref. 82). The recently published Food and Drug Administration regulation requiring designation of whether a unit has been obtained from a paid donor is directed at this risk factor.

In recent years, studies have been increasingly directed at elucidating the etiological agent(s) responsible for post-transfusion hepatitis unrelated to the Hepatitis B agent. While hepatitis A may be involved in some cases, it is clear that there are a large number of "non-A and non-B" cases which currently escape detection for lack of appropriate tests. Development of these test must be encouraged (Refs. 80, 81, 183 and 186).

New lines of investigation hold promise for improved control of Hepatitis B infection. Studies on the core antigen, HBcAg, and its antibody, the e antigen and DNA polymerease hold promise for better understanding of Hepatitis B and perhaps ascertainment of which units are truly infectious.

No changes are recommended at this time regarding hepatitis testing of stored red blood cell products.

14. *Labels for blood bags—a. Content and design.* The label affixed to the blood bag must be designed to enhance the safety of the product by providing adequate vital information to all individuals who handle it. Unnecessary information, or information that is readily available elsewhere, when present on the label, may interfere with optimal blood bank practice by distracting the eye from the vital information. As it is generally agreed that most avoidable serious complications of blood transfusion result from "clerical" rather than technical errors, the information present on the label and the manner in which it is displayed assume major significance.

Labels currently affixed to units of whole blood or red blood cells contain much information which is almost never read or used. Much of the information documents that the units have been processed in a manner which meets Federal regulations. As blood may not be collected or transfused otherwise, the presence on the label of such information is unnecessary and detracts from the safe use of the product. However, this information should be available in every blood bank as part of the product labeling.

b. *Color coding of labels.* Currently, color codes for blood typing labels are optional. While there are no data to indicate whether color coding of labels for ABO blood types would help reduce blood transfusion errors, the Panel feels that immediate recognition of blood types by label color would be advantageous, particularly in acute, massive transfusion situations.

c. *Recommendations for blood bag labels.* The following information on labels affixed to units of Whole Blood (Human) or Red Blood Cells (Human) is vital to the safety and effectiveness of the product and must appear on the bag with the first four items in greatest prominence:

(1) Name of the product including designation of the anticoagulant-preservative solution. The designation "(Human)" is unnecessary.

(2) Blood type. ABO and Rh (Rh_oD positive or negative) types of blood are of vital importance and must be displayed most prominently.

(3) Expiration date.

(4) A unique number which will permit tracing the unit of blood back to the donor. The number used to identify the unit of blood must be the only prominent number.

(5) Manufacturer's codes, referring to codes of blood bag manufacturers including lot numbers have no intrinsic value in routine use and should be displayed on the label in an unobtrusive place in small print.

(6) The name of the blood collection facility should be on the label in an as unobtrusive manner as possible.

(7) The designation that the unit was collected from a "Volunteer Donor" should be discontinued. The designation "Paid Donor" should be retained and used when appropriate.

(8) Instructions:

(a) Store between 1 to 6 °C.

(b) Do not add medication other than 0.9 percent NaCl solution.

(c) Transfuse through a filter.

(9) The following statement, "For additional information refer to circular of information."

(10) Space for additional labeling. If the unit has special properties, e.g., donor antibody or an unusual red cell phenotype, this should be noted on the label in an available space.

(11) License number in a small and unobtrusive manner.

(d) *Other label recommendations.* (1) Blood labeling regulations should be modified to require that those items listed above, and only those items, be present on all units of Whole Blood and Red Blood Cells as delivered by the collecting agency.

(2) Research should be encouraged for analysis of the best eye-readable format for blood bag labeling.

(3) The developing of machine-readable costs on labels must be conducted in a way to assure that the results would not detract from the eye-readable aspects of the label.

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4. Whole Blood (Human) Heparin

Generic Statement

1. *Introduction.* The commonly used anticoagulant-preservative solutions for collection of blood contain citrate to provide a calcium binding effect to prevent clotting. There are clinical situations in which citrate might adversely affect the availability of ionic calcium in the blood of the recipient (see the section on anticoagulants in relation to whole blood). In addition there is an acid load of up to 30 to 40 mEq per liter in stored citrated blood. The excess acidity is due mainly to the citric acid in the anticoagulant and the lactic acid generated during storage (see section on acid-base alterations of whole blood). For these reasons, other substances have been considered for use as anticoagulants.

Heparin is a sulfated mucopolysaccharide that acts with a co-factor antithrombin III and is a potent anticoagulant (Ref. 1). The co-factor is present in adequate amounts in normal donor plasma so that the addition of heparin alone will provide the desired anticoagulant effect. Heparin appears to act at more than one point in the sequence of coagulation in that it appears to inhibit the conversion of prothrombin to thrombin and also inhibits the action of thrombin on fibrinogen (Ref. 1).

Heparin is not a suitable anticoagulant for long-term storage of blood. With time, its effects are neutralized by normal constituents of blood (Ref. 1). In addition, it is not a preservative of red blood cells (Refs. 2 through 4). The amount needed as an anticoagulant for human blood intended for transfusion is 75,000 units of Heparin Sodium (U.S.P.) with sufficient Sodium Chloride Injection (U.S.P.) to make 1,000 mL. Six mL of this solution as used for each 100 mL of whole blood (Ref. 6) will provide adequate anticoagulant effect for no more than 48 hours. Plasma separated from heparinized blood is not suitable for use as Single Donor Plasma (Human) or as a starting material for preparation of Cryoprecipitated Antihemophilic (Human) Factor or platelets.

2. *Analysis of indications and labeling*—a. *Indications.* This product is indicated when there is a need to provide both red blood cells and plasma and the administration of a citrate is contraindicated. Depending on the clinical situation, heparinized blood may be indicated in exchange transfusions of newborn infants, when large amounts of blood are required in patients with liver disease and who are unable to metabolize citrate rapidly and in cardiopulmonary surgery with extracorporeal circulation.

b. *Evidence of effectiveness.* It is not possible to find detailed controlled studies which establish the effectiveness of Whole Blood (Human) Heparin. There is anectodal information that it has been used, and no discernable adverse effects have resulted. Current regulations require that heparinized blood be used within 48 hours of collection. It appears that the red blood cell viability is not adversely affected by short-term storage, but the viability of red blood cells in vivo after storage of heparinized

blood has not been well documented. There is limited evidence to support the current dating time of 48 hours or to document other changes that occur in heparinized blood during storage (Refs. 2 through 5).

c. *Evidence of safety.* Heparinized blood has been used for a variety of reasons, and no documented evidence is available to indicate that when used appropriately it is unsafe. When large amounts of heparinized blood are administered, there may be sufficient heparin infused to result in a hemostatic defect in the recipient. Heparin in relatively large amounts is used in the treatment of thrombin-embolic diseases. Even with therapeutic dosages, reactions to heparin administered intravenously ae rare other than for its effect on blood coagulation and hemostasis.

Heparinized whole blood does not contain the added glucose that is present in ACD or CPD anticoagulants. This must be kept in mind in patients receiving heparinized blood in exchange transfusions.

d. *Benefit/risk evaluation.* As with all human blood products, there are risks associated with this product. Except for the possibility of coagulation or hemostatic problems resulting from the amount of heparin transfused, the risks are the same as those discussed in the section on whole blood. There are clinical situations when use of blood of a more neutral pH and which does not contain a calcium-binding anticoagulant is indicated. For this reason Whole Blood (Human) Heparin should continue to be available.

e. *Recommendations.* (1) Whole Blood (Human) Heparin should be placed in Category III A with a period of 3 years allowed for completion of further studies. The studies should include red cell survival data, the status of the coagulation system in the donor blood, activator levels, degree of kinin activation, the presence of clots, and residual heparin.

(2) Although no adverse effects have been reported following transfusion of heparinized blood stored up to 48 hours, it appears wise to limit storage of such blood to no more than 8 hours prior to use. If the product is to remain licensed during the period of additional studies, the expiration time should be reduced to 8 hours after the initial drawing of the blood.

(3) The general labeling requirements applicable to blood collected in ACD or CPD apply except for those data related to anticoagulation and red cell preservation. The labeling should also indicate that heparinization of recipients is a real possibility when multiple transfusions of heparinized blood are given.

(4) It has been the practice in some institutions to add ACD or CPD anticoagulant to heparinized blood and then to use the blood up to 12 days after collection (Ref. 5). This is not a procedure discussed in the Code of Federal Regulations. If there is a need for such a modification, additional studies will be needed to demonstrate the safety and effectiveness of the product.

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5. Whole Blood (Human) Modified-Antihemophilic Factor Removed and Whole Blood (Human) Modified-Platelets Removed

Generic Statement

1. *Introduction.* Blood collected into interconnected but separable plastic containers containing a suitable anticoagulant may be modified without contaminating the unit. Platelets may be separated from the whole blood by selective centrifugation and transfer of plasma along with most of the platelets to an integrally attached compartment. The platelets can then be concentrated by centrifugation and most of the platelet-poor plasma can be returned to the compartment containing the red blood cells. This modified blood has the characteristics of the starting whole blood except that the majority of the platelets and approximately 50 mL of plasma have been removed.

In a similar manner, Cryoprecipitated Antihemophilic Factor (Human) (cryoprecipitate) can be prepared while the containers are still integrally attached and the cryoprecipitate-poor plasma can be returned to the compartment containing the red blood cells. In this procedure plasma is separated from whole blood and transferred to an integrally attached compartment. The plasma is processed to yield the cryoprecipitated antihemophilic factor while the multicompartmented container remains attached. During the processing, the red blood cells are maintained at 4 to 6 °C. The cryoprecipitate is harvested, and the residual plasma can be returned to the compartment containing the red blood cells. As indicated in the section on Cryoprecipitated Antihemophilic Factor (Human), the residual plasma contains 15 to 35 percent of the original Factor VIII activity, about ⅓ of the original fibrinogen and approximately 90 percent of the original Factor V activity. The activity of other coagulation factors are unchanged from those of fresh plasma. The modified whole blood has the characteristics of whole blood except that most of the Factor VIII, ⅔ of the fibrinogen, and some of the Factor V have been removed.

The two processes can be combined so that both platelets and Factor VIII are removed. It is generally considered to be more practical to seal and detach the compartment containing the red blood cells before proceeding with separation of platelets or cryoprecipitate. In this way, Red Blood Cells (Human) are prepared in the usual way and the processing for platelets and/or cryoprecipitated antihemophilic factor is done independent of the Red Blood Cells (Human).

2. *Effectiveness.* The indications for the modified whole blood products are the same as for whole blood stored for more than 48 hours. When used in this manner, the effectiveness is equivalent to that of stored whole blood for red cell and plasma volume replacement.

3. *Safety.* The safety of the product is equivalent to that of whole blood. The manipulations done do not appear to have any adverse effects.

4. *Benefit/risk evaluation.* The same considerations apply as for whole blood. In most blood bank estabilishments, the red blood cells will be removed prior to processing for cryoprecipitate and/or platelets. In a few situations with unique requirements, this is a useful product. Because of the hepatitis risk, this product may be safer than providing red blood cells and plasma from two separate donors. However, there are no advantages in using this product in comparison to whole blood.

5. *Recommendation.* It is recommended that these products be placed in Category I, safe and effective.

6. *Labeling analysis.* The labeling requirements for whole blood apply to these products. In addition, the container labels must indicate clearly that platelets and/or cryoprecipitated antihemophilic factor have been removed.

6. Red Blood Cells (Human)

Generic Statement

1. *Introduction.* Red Blood Cells (Human) are also called Concentrated Red Blood Cells by the World Health Organization. The other designation of this whole blood component as "packed red blood cells" usually means red blood cells from which most of the plasma has been removed either by sedimentation or by centrifugation. The term can also include centrifuged cells with the buffy coat largely removed or washed red cells in which plasma and buffy coat have been removed by at least three washings. Thus, four products may be available to the clinician, each with somewhat different characteristics (Ref. 1).

Sedimented red blood cells are the most easily available type since no mechanical equipment is needed to prepare them. Sedimented cells contain practically all of the leukocytes, platelets, and almost the same concentration of potassium (less than 1 mEq difference) in the residual plasma as in the plasma of whole blood of equivalent age (Ref. 2).

Centrifuged red blood cells have similar characteristics, but permit the harvesting of more plasma, yielding a hematocrit of about 60 to 80 percent. They are more viscous because of the high hematocrit.

2. *Indications.* Red Blood Cells (Human) are administered to increase the oxygen-carrying capacity of the blood. This indication was discussed earlier in the statement on "Stored Red Blood Cell Products". Patients with hypoxic symptoms whose anemia is unresponsive to specific treatment: e.g., iron, B_{12}, folic acid, etc., who are not hypovolemic and who do not require plasma-clotting factors or plasma proteins may be efit from administration of red blood cells (Refs. 9 and 10). Red blood cells should not be used in cases in which whole blood is indicated for treatment of hypovolemia, unless accompanied by the administration of plasma volume expanders. Plasma volume may be expanded, at least in the face of moderate degrees of acute blood loss, by administration of crystalloid solutions.

3. *Effectiveness.* Extensive experience and research has proven the effectiveness of administering Red Blood Cells (Human) to increase the oxygen-carrying capacity of the blood (Ref. 8). Stored red blood cells, whether concentrated or not, have an increased affinity for oxygen. Because red blood cells are transfused to provide patients with adequate oxygen transport, it has been suggested that blood with high oxygen affinity is undesirable and that only blood with nearly normal oxygen affinity is suitable for transfusion. However, available data do not indicate the extent of the benefits that might result from blood cells with nearly normal oxygen affinity or the costs and risks that might be incurred in obtaining products to yield such possible benefits (Ref. 11). Red blood cells are the material of choice for transfusion of the anemic patient with limited cardiovascular reserves.

4. *Safety.* The hazards of red blood cell transfusions are the same as whole blood transfusions. The only hazards not associated with whole blood transfusions are possibly those of ammonia accumulation and citrate intoxication (Ref. 12). Since potassium from the red cells equilibrates with the plasma potassium as storage progresses, red blood cells are only slightly deficient in potassium (Ref. 2).

In general, it has been stated that fewer transfusion reactions occur with red blood cells compared with whole blood, but it should be emphasized that this is in reference to "buffy coat poor" red blood cells or previously frozen, washed cells. These latter two products are quite different from the red blood cells available in most blood banks. In one report, 59 percent of transfusion reactions were caused by red blood cells when 48 percent of all red blood cell products were given as red blood cells (Ref. 6). The reduction in plasma volume of sedimented red cell products which have a hematocrit of 65 to 70 percent is insufficient to prevent IgA reactions in sensitive recipients (Ref. 3) or hepatitis (Ref. 4). The deleterious effects of allergic reactions and anaphylactic reactions to IgA are obviated only by washed red cells (Ref. 5). Centrifuged red blood cells with the buffy coat largely, but not completely, removed contain about 30 percent of the leukocytes present in the original donor unit. Sufficient leukocytes and platelets are still present to cause allosensitization to their antigens in the previously unsensitized patient.

The 5 to 10 percent residual plasma in centrifuged red blood cells is too high to provide any protection against the development of hepatitis (Ref. 1). There has been no definitive prospective analysis to substantiate the claim that the incidence of hepatitis with red blood cells is less than that of whole blood.

Washed red blood cells are indicated for patients who have allergic transfusion reactions. Patients who develop anaphylactoid reactions to IgA must receive red blood cells almost completely free of plasma. Even the small amount of plasma (less than 1 percent) that remains in well washed red blood cells can cause transfusion reactions in some of these patients (Ref. 3).

5. *Benefit/risk evaluation.* This product allows the use of red blood cells as a byproduct of the preparation of other blood components, and it is completely adequate to treat the deficiency in red blood cell oxygen-carrying capacity associated with anemia. Red blood cells are difficult to administer rapidly and may be more expensive than whole blood if they must be administered with plasma substitutes to replace blood volume loss (Ref. 8). Sixty percent of the blood collected is used intraoperatively (Ref. 7).

6. *Recommendations.* a. Red Blood Cells (Human) should be placed in Category I.

b. The term "Red Blood Cells" should be substituted for "Red Blood Cells (Human)" and for "packed red blood cells".

c. Red Blood Cells should be labeled to indicate that their only use is to increase the oxygen-carrying capacity of the blood. When they must be used to substitute for Whole Blood then the labeling must indicate that a suitable plasma volume expander must be administered with them if hypovolemia is being treated (Ref. 13). In other situations where whole blood is indicated because of its red blood cells and plasma constituents, red cells can be used with simultaneous administration of the necessary plasma components in a separate form.

d. Labeling should indicate that red blood cells should not be reconstituted or infused with lactated Ringer's solution, 5 percent aqueous dextrose or 5 percent dextrose in 0.225 percent saline, as clumping, hemolysis, or clotting may occur (Ref. 14).

e. There is a need for the scientific community to develop a more extensive evaluation of the usefulness of red blood cells and an analysis of the complications that occur after their administration. This is especially relevant to the belief that the administration of Red Blood Cells will minimize circulatory overload.

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7. Frozen Red Blood Cells (Human)

Generic Statement

1. *Introduction.* The development of methods for cryopreserving blood was predicated on the perceived need for long-term storage. Vagaries in blood collections, daily and seasonal, as well as uncertainties in usage contributed to episodic cycles of blood shortages followed by excesses and, thus, outdating. Prolonged extension of the blood's shelf life was seen as a possible way of circumventing these variables and making more effective use of collected blood.

Expansion of techniques for antibody identification and the description of new red cell phenotypes during the past two decades also resulted in the need for long-term storage. Patients with antibodies directed against high incidence antigens require a ready resource of homologous or autologous blood. This could be accomplished by extending the shelf life of blood indefinitely.

A number of techniques for red cell cryopreservation have been developed and are in routine use (Refs. 1 through 4), but only two have been licensed by the Food and Drug Administration (Refs. 1 and 3). Although methods vary considerably, they have one common major requirement: The necessity of adding the cryoprotective agent, glycerol, prior to freezing. Since the intravenous administration of large amounts of glycerol is undesirable, previously frozen, glycerol-protected blood must be washed prior to transfusion. This is also required to return the cells to an isosmotic state, since glycerol penetrates the red cells in exerting its cryoprotective effect.

The cost of washing, in both dollars and time, has restricted severely the routine use of previously frozen red cells of purposes of balancing inventory. Furthermore, techniques of freezing and washing red cells require multiple entries into the blood pack, thereby restricting the post-thaw shelf life to a mere 24 hours according to current practice. However, an impressive number of other possible advantages resulting from either freezing and/or thawing have come to light which appear to be separate from the long shelf life of frozen red cells but which at this time play a greater role in their clinical use. Verification of these presumed benefits of frozen red cells might well determine the extent of their use in the near future. Despite these considerations, it is estimated, based on data accumulated within The American Red Cross Blood Program, that no more than 3 to 5 percent of all blood transfusions are currently accomplished with frozen red blood cells.

2. *Effectiveness*—a. *Red cell studies.* The recovery of red cells after glycerolization, freezing, thawing and deglycerolization (washing) varies between 70 to 93 percent of the original starting material and depends largely on the method used for the freeze-thaw procedure (Refs. 3 and 27). Most studies report that at least 85 percent of the red cells are recovered when a high-glycerol technique is used and if deglycerolization is done using one of the three currently available centrifuge techniques. More controversy exists in evaluating the technique of cytoagglomeration where some investigators report recoveries as low as 70 percent, although others have found 85 percent recovery (Refs. 1 and 27).

Analysis in vitro of the final deglycerolized product comparing various washing technique has been performed by Valeri (Ref. 28). He found that the red cell products obtained by the cytoagglomeration techniques had higher levels of supernatant hemoglobin, extracellular potassium ion, supernatant osmolality, residual glycerol, and residual plasma protein than did cells washed by any of the currently available centrifuge methods, all of which seem to give comparable results for these measured characteristics.

Measurements of survival of deglycerolized red cells 24 hour after transfusion demonstrate differences depending on the method employed in freeze-thawing and the duration of storage in the frozen sate (Ref. 29). For the cytoagglomeration technique, in one study of 18 units stored frozen for a mean of 3.8 ± 1.5 years, a mean 24-hour survival of 73 percent plus or minus 17.5 (2 S.D.) was found. It has also been demonstrated that with this method there is a correlation between the duration of storage in the frozen state and the 24-hour red cell survival (Ref. 4). When using one of the centrifugal methods for deglycerolization, higher 24-hour survivals are usually obtained, especially in direct comparative studies with the cytoagglomeration method. Furthermore, the results obtained by these methods do not seem to be affected by duration of frozen storage.

When using the cytoaggolmeration technique it has been found that nearly 50 percent of all units have a positive direct antiglobulin test. This finding, however, could not be correlated with any alterations in the 24-hour red cell survival (Ref. 30).

Frozen deglycerolized red cells require a two-faceted evaluation: the recovery of cells in vitro after freeze-thaw injury and the survival of these cells after transfusion. These characteristics are of importance to the recipient as they relate directly to the expected benefit after transfusion of "one unit." As there is red cell loss during the freeze-thaw process which is related to variations in methods used for freeze-thawing, as well as variations in post-transfusion recovery related to the same methods, each technique could result in wide variations in therapeutic effectiveness of the resulting red cell product.

b. *Possible advantages of frozen blood.* Table 1 lists the possible advantages of frozen-deglycerolized red cells.

TABLE 1.—POSSIBLE ADVANTAGES OF FROZEN RED CELLS

	Freezing	Washing
Leukocyte removal	?	+
Removal of hepatitis B antigen	0	?
Plasma protein removal	?	+
Diphosphoglycerate maintenance	+	0
Long-term storage of rare types	+	0
Salvage of outdated blood	+	+
Inventory control	+	-
Microaggregate removal	0	+

? Unknown; + Contributes an advantage; 0 Probably unrelated to an advantage; - Deleterious.

In each case in Table 1 the role of either the freezing or thawing processes is assessed as being contributory, unrelated, or deleterious to the desired result. In many cases, the washing procedure appears to be far more important than freezing. This suggests that washing alone without prior freezing may be sufficient to achieve many of the proposed advantages of frozen red cells. Thus, the efficiency of washing is critical in determining some of the possible advantages of frozen red cells. Variables in washing techniques, such as wash volume or whether batch or continuous flow washing is used, may determine how well suited a particular method is for achieving a desired property of the red cell preparation such as leukocyte or hepatitis agent removal. A corollary is that efforts to reduce the time and expense of the washing procedure might result in a loss of some of the possible advantageous aspects of frozen red cells. The limit of effective washing of red cells has not yet been determined.

(1) *Leukocyte removal.* For several transfusion situations, contamination of red cell preparations with leukocytes is undesirable. There are four problem areas in which the presence of leukocytes appears to play a major role: (1) Nonhemolytic febrile transfusion reactions are largely the result of immunization to leukocyte and/or platelet antigens. (2) Refractoriness to platelet transfusion has been correlated with previous transfusions with leukocyte and platelet-containing blood products. The use of previously frozen red blood cells may minimize this problem. (3) Immunization to leukocyte and platelet antigens of the HLA and other systems by transfusion may compromise the survival of future allografts, although this concept has recently been brought into serious doubt. (4) Leukocyte-borne viruses may cause disease in recipients. In addition to these problems, the transfusion of viable lymphocytes to immunosuppressed recipients has been suggested to be hazardous by virtue of causing graft versus host disease.

Compared with liquid-stored blood, frozen-washed red cells have few leukocytes and virtually no platelets. There is considerable disagreement over the extent of leukocyte removal achieved by this method. Table 2 indicates the extent of red cell losses and leukocyte reduction achieved with some current freeze-thawing techniques (Table 2). It appears that the freezing process may play an important role in Facilitating efficient leukocyte removal by simple destruction since current methods for cryopreserving red cells are not effective for preserving leukocytes or platelets. Some studies suggest that some lymphocytes may be resistant to the rigors of this method of cryopreservation (Ref. 13). Undoubtedly, the necessary post-thaw washing also plays a role in leukocyte removal. Despite this, more complete removal of HLA antigenicity may require micropore filtration during transfusion (Ref. 14). Leukocyte removal also can be achieved without freezing using a variety of separation techniques (Table 2).

TABLE 2.—RED CELL LOSSES AND LEUKOCYTE REDUCTION ASSOCIATED WITH VARIOUS CRYOPRESERVATION METHODS

Method	Percent RBC loss	Percent leukocyte reduction	Reference No.
Freezing Procedures:			
Freezing (cytoagglomeration) (ACD)	37	84	21
Freezing (cytoagglomeration) (CPD)	27	61	
Freezing (centrifugal) (fresh cells)	5	93	17
Other Procedures:			
Inverted spin	24	80	25
Inverted spin plus continuous flow wash	42	80	
Dextran sed. plus continuous flow wash	35	92	
Inverted spin	21	73	19
Upright spin	20	72	
Nylon filter	12	65	
Nylon filter then inverted spin	32	97	
Upright spin, then nylon filter	40	97	
Upright spin, then inverted spin	22	87	
Dextran sedimentation	8	93	22
Plasmagel sedimentation	6.5	93.5	

Regardless of the underlying mechanism, the need for transfusions of leukocyte-poor blood is currently the major indication for using frozen-washed red cells.

Frozen-washed red cells appear to be effective for patients who have been transfused frequently and who have had repeated febrile reactions due to antibodies to transfused leukocytes. In some transfusion services frozen-washed red cells are used prophylactically for patients in whom long-term transfusion therapy is anticipated. Most users of frozen blood for this purpose have noted an almost complete absence of nonhemolytic, febrile transfusion reactions but published data on this point are lacking. These clinical observations have been supported by preliminary studies on the production of cytotoxic and agglutinating luekocyte antibodies following frozen blood transfusion.

Probably the most common use of frozen blood is in patients with chronic renal disease either on a dialysis program or as part of a renal transplant procedure. Approximately 50 percent of all frozen red blood cell transfusions in the Red Cross program are in this category. Here the use of frozen cells is predicated on their presumed lack of immunogenicity in the HLA system because few leukocytes are present in the final transfusion product. It is postulated that the rejection rate of a renal homograft could be diminished with the use of frozen red cell transfusions. Conversely, results from a retrospective, multicenter study have suggested that transfusion might actually be protective against renal allograft rejection possibly by stimulating the production of "blocking" antibodies (Ref. 5). More recent studies indicate that homograft survival is in fact better in recipients previously transfused with liquid stored blood containing leukocytes than in nontransfused recipients (Refs. 31, 32, 33, 39 and 40). These studies bring sharply into question the current general use of this blood product for this indication.

(2) *Risk of hepatitis.* The report of Tullis et al. (Ref. 6) on the absence of hepatitis in recipients of previously frozen blood was a major force in accelerating development of freeze-thaw technology and in the clinical use of this blood product. Another retrospective study confirms this observation (Ref. 34). In a limited prospective study, Valeri (Ref. 35) demonstated that washing blood prior to transfusion reduced the infectivity rate for Hepatitis B. Recent studies indicate, however, that HBsAg infectivity can be transmitted to chimpanzees transfused with frozen deglycerolized red blood cells washed by continuous flow techniques (Ref. 37). More extensive controlled studies on this question are necessary to quantify this effect.

It has been demonstrated that Hepatitis B surface antigen (HBsAg) cannot be detected even by the most sensitive techniques in blood thoroughly used by either a batch process or by continuous flow (Ref. 7). The latter technique, which can achieve a plasma protein dilution of 1:25,000, produce a 40-

fold greater protein dilution than triple washing in a batch process. Therefore, continuous flow might be the preferable method, particularly since HBsAg can be passively transferred to recipients even if it is transfused at great dilution. This question may be crucial since freeze-wash methods that utilize batch washing procedures may be at a theoretical, if not a real, disadvantage (Ref. 35). This difficult question requires very careful study.

(3) *Plasma protein removal.* Frozen-washed red cell preparations are largely devoid of plasma protein. Thus, transfusion reactions resulting from previous immunization to plasma proteins such as anaphylaxis due to sensitivity to IgA, or from passively administered antibodies such as those to penicillin may be eliminated by using frozen blood. Freezing may play some role by facilitating the removal during the washing procedure of some proteins previously bound to the red cell surface. A patient with sensitivity to IgA was transfused safely only with blood that was both frozen and washed but not with blood without prior glycerolization and freezing (Ref. 8).

The absence of anti-A and anti-B alloagglutinins in freeze-washed red cells makes this product safe when used judiciously in recipients of other ABO groups, Group O to any recipient, Group A or B to AB recipients. This is helpful from the point of view of the transfusion service because it permits greater flexibility in selecting compatible blood. Also, units of blood containing unexpected antibodies can be diverted into the frozen blood inventory. The antibodies present in these units are then removed during the deglycerolization-wash procedure.

(4) *Maintenance of red cell diphosphoglycerate (DPG).* Much recent research has been directed towards developing methods for maintaining DPG levels and oxygen-releasing capability in stored erythroocytes. Red cells stored in the frozen state show no diminution of DPG levels after thawing when compared with pre-freeze levels (Refs. 9 and 10). Since most blood is frozen soon after collection, DPG levels are maintained at levels approaching those of freshly drawn blood. Red cells with normal DPG levels may have some applications in special transfusion situations, but more carefully designed and controlled studies are necessary to establish this point (Ref. 12).

(5) *Long-term storage of rare blood types.* Freezing permits long-term storage of red cells which are negative for various high-incidence antigens or other uncommon antigen phenotypes for future transfusion into immunized recipients. This is an important but uncommon application of cryopreservation, which represents less than 5 percent of all frozen blood usage in the Red Cross Blood Service.

(6) *Salvage of outdated blood.* Valeri and Zaroulis have demonstrated recently that blood collected in ACD or CPD and stored at 4° C for up to 28 days can be "rejuvenated" by incubation at 37° C for 1 hour with pyruvate, inosine, glucose, and phosphate (Refs. 10 and 11). Subsequent freezing, thawing, and washing yielded an 86.6 percent in vitro recovery of red cells having a 24-hour post-transfusion survival in vivo of 80 percent. The oxygen transport function of these cells was the same as that of freshly collected blood. Post-thaw washing was necessary to remove the potentially toxic materials used for rejuvenation. This technique offers a possible new approach to control of red cell outdating. Rejuvenation and freezing may play some role where salvage of specially selected red cells such as those of uncommon phenotype is necessary.

(7) *Removal of microaggregates.* It is generally assumed that the majority of microaggregates in stored blood are removed during the washing procedure although data establishing this point are lacking. A recent study suggests that some microaggregates remain which can be removed by micropore filtration (Ref. 14).

3. *Safety.* In general, most of the hazards related to liquid-stored whole blood or red blood cells apply to frozen deglycerolized cells as well. Some of the areas where it has been well established that frozen deglycerolized red cells have higher levels of safety include: treatment of patients with previous nonhemolytic febrile transfusion reactions, treatment of patients with allergy to plasma proteins, in decreasing immunization to leukocyte or platelet antigens where that may be desirable, in maintenance of red cell DPG levels, and in non-ABO specific transfusions where alloantibody removal is desirable. Increased safety related to hepatitis transmission has not been proven. Use of frozen blood in hemodialysis patients for improving later allograft survival now seems questionable.

The freeze-thaw process itself adds certain potential hazards. Adequate removal of glycerol must be achieved. The ionic constitution and concentration of the final wash solutions may be of some concern if they do not approach normal plasma levels in the final preparations. The possibility of bacterial contamination of the final product because of its preparation in an open system is an additional hazard. The current 24-hour post-wash shelf life for frozen deglycerolized blood is consistent with similar guidelines for other blood preparations prepared in open systems. In one study of this issue, units of whole blood were inoculated with a variety of bacteria prior to freezing or after deglycerolization. These units were evaluated for bacterial contamination after postdeglycerolization storage for 72 hours at 4 °C (Ref. 36). It was demonstrated that, (a) the deglycerolization procedure reduces the bacterial inoculum substantially and (b) the bacteria evaluated in this study do not proliferate in the deglycerolized red cell product for up to 72 hours when properly stored. It was later noted that one cryophillic bacterial species did proliferate under these conditions.

4. *Recommendations.* a. The Panel recommends that Frozen Red Blood Cells (Human) be placed in Category I.

b. Frozen deglycerolized red blood cells must be prepared by a method that results in a minimum in vitro yield of 80 percent of the original red cell mass.

c. Frozen deglycerolized red blood cells must meet the same viability requirements as liquid stored blood, namely, the method must be designed to assure that, on the last storage day, at least 70 percent of the cells survive in the recipient 24 hours after transfusion.

d. The effective yield after deglycerolization must be 65 percent. The effective yield is determined by multiplying the mean in vitro yield after deglycerolization by the mean percentage in vivo survival 24 hours after transfusion.

e. If the above criteria are met and if it can be shown that there is no increased risk to the recipient of bacterial contamination when compared with liquid-stored blood prepared in a closed system, the post-deglycerolization shelf life should be extended beyond the presently permitted 24 hours.

f. Glycerolized blood may be stored in the frozen state for any period of time as long as it has been demonstrated that the above criteria are met.

g. After deglycerolization, the final suspending solution should be isotonic and contain between 130 and 160 mEq sodium per liter.

h. In-process controls of the freezing and deglycerolization procedure should be such as to assure that there shall be no more than 0.5 gram per dL of glycerol and 175 mg per dL of supernatant hemoglobin in the final product (Ref. 38).

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8. Cryoprecipitated Antihemophilic Factor

Generic Statement

1. *Introduction.* In 1954 Brinkhous (Ref. 1) first made the observation that factor VIII (antihemophilic factor, AHF) was a cold insoluble protein. In 1959, Pool and Robinson (Ref. 2) made a similar observation while studying plasma preparations of factor VIII. However, it was not until 1965 that Pool and Shannon (Ref. 3) described a method for the practical utilization of factor VIII cryoprecipitability to prepare cryoprecipitated Antihemophilic Factor (Human) ("Cryoprecipitate", "Cryo") as a part of routine blood banking. The procedure involves freezing plasma with subsequent thawing at 4° C yielding a precipitate containing about 45 percent of the original factor VIII and 30 to 40 percent of the fibrinogen. After centrifuging, the supernatant plasma is drained and the residual cryoprecipitated factor VIII is refrozen. It is a simple, inexpensive way to provide factor VIII as a byproduct of component blood banking. The major problem has been the unpredictable yield of factor VIII making dosage calculations difficult (Ref. 4). However, recent studies (Refs. 5 and 6) have analyzed the variables of production which may affect yield. This has given a standardized method which consistently provides an average of 60 percent of the available plasma factor VIII in the cryoprecipitate. In addition to Cryoprecipated Antihemophilic Factor (Human), commercial Antihemophilic Factor (Human) has subsequently been licensed and is reviewed elsewhere in this document.

2. *Factor VIII assay.* The two major techniques of assaying factor VIII are based on the partial thromboplastin time (PTT) (Ref. 7) or thromboplastin generation time (TGT) (Ref. 8). These methods originally were developed for measuring factor VIII levels in plasma. One hundred percent activity was based on an appropriate-sized normal donor pool (10 to 30 donors) or the then-available NIH reference standard. These assays were shown to predict the recovery of factor VIII when plasma was transfused into hemophilia A recipients. Although the assays correlate with each other to a high degree, both have a variability within the assay system of 7 to 10 percent. The increased concentration of factor VIII in more recent cryoprecipitate procedures has required modification of the assay systems to give reliable measurements. The major change suggested for both assays (Refs. 3 and 9) was an initial dilution of cryoprecipitate 1 to 9 in hemophiliac plasma with further dilutions in buffer to give a factor VIII concentration within the range of the standard assay curves; i.e., 0 to 100 percent activity. In spite of these modifications the assays may not predict the recovery in vivo (Refs. 3 and 5). However, studies comparing the two methods for measuring factor VIII in cryoprecipitates have shown that they are comparable (Refs. 5 and 10). Because of the often unexplained differences between in vitro factor VIII assays and in vivo factor VIII recoveries (Refs. 3, 5 and 11 through 15), facilities preparing Cryoprecipitated Antihemophilic Factor (Human) should document the reliability of the in vitro assay system used by measuring in vivo recoveries of factor VIII following the infusion of their cryoprecipitate into hemophilia A recipients (Refs. 3 and 5).

3. *Donor variables.* Several different characteristics of the donor have been claimed to be associated with higher plasma factor VIII levels and presumably a greater yield of factor VIII in cryoprecipitates prepared from this starting plasma.

a. *Factor VIII levels in the normal population.* It has consistently been shown that the normal population has a wide range of factor VIII activity from 50 to 200 percent and that this level may not even be the same for a donor on every occasion (Refs. 7 and 16 through 19). The greater the factor VIII activity of the initial plasma the greater the expected yield of factor VIII in cryoprecipitate. However, some studies have suggested that not all donors have the same degree of cryoprecipitability (Refs. 6 and 17). Nevertheless, this baseline factor VIII variability in the donor is probably the major factor in the spread of factor VIII activity in individual units of cryoprecipitate.

b. *Exercise.* Exercise has been shown to increase the factor VIII concentration in plasma 1½ to 3 times normal (Refs. 14 and 20 through 25) and in those studies where it has been evaluated an increase in factor VIII antigen has also been found. The immunologic data suggest that the increase in procoagulant activity represents a true elevation of factor VIII protein.

However, transfusions of plasma from exercised donors into hemophiliacs have shown a decreased factor VIII recovery (Ref. 20) and shortened half-life of 5 hours compared to the normal of 10 to 13 hours (Ref. 22). In vitro assay of cryoprecipitates prepared from exercised plasma donors over-estimated the in vivo yield (Ref. 14). Therefore, exercising donors does not represent a useful means of increasing the production of factor VIII.

c. *Other variables.* Age, sex, and blood group are other possible donor variables. Preston (Ref. 16) found factor VIII activity to be 6.5 percent greater in females ($p < 0.05$), 8 percent greater in A or B blood group donors than those of group O ($p < 0.01$) and unaffected by the age of the donor in an analysis of plasma from 410 individuals. In contrast, similar studies by Burka (Ref. 17) in 169 donors showed no significant differences in any of these parameters.

4. *Preparation variables*—a. *Sources.* Either single units of whole blood or plasmapheresis may be used as a source of starting plasma. Some studies have reported an averaged 15 percent reduction in factor VIII activity in the second unit of a double plasmapheresis (Refs. 17 and 26), while others have found no differences (Refs. 5 and 6). The reported differences in plasma factor VIII levels between plasmapheresis units are small enough that they do not statistically affect factor VIII yield in cryoprecipitates (Ref. 17).

b. *Collection*—(1) *Anticoagulant.* The anticoagulant used, whether ACD or CPD, does not affect the yield of factor VIII in cryo (Refs. 5, 17, 27, and 28) nor does the addition of adenine (Ref. 29). Factor VIII blood is mixed thoroughly at the end of collection. Factor VIII content is most stable at pH values greater than 6.8 (Ref. 28) and preparation of cryoprecipitate from ACD plasma with a pH of 6.5, as may occur after preparation of platelet concentrates, is associated with a 43-percent average reduction in the in vivo factor VIII yield (Ref. 11).

(2) *Container.* The collection container, plastic or glass, does not affect AHG yield (Refs. 5, 18, and 19).

(3) *Mixing during collection.* Perkins (Ref. 19) found a significant improvement in factor VIII levels in blood collected by a rocking vacuum device compared to gravity blood flow. Plasma Factor VIII levels were better in fresh plasma with approximately 98 percent of expected levels with the vacuum method versus 80 percent by gravity flow ($p < 0.05$). Similar differences were found after frozen storage of the plasma for 2 months. Subsequent studies, however, have shown that as long as the blood is mixed thoroughly at the end of collection, factor VIII content of cryoprecipitate does not differ significantly from that prepared from blood agitated throughout the collection (Refs. 5 and 6).

(4) *Delay before processing.* Whole blood stored at 4° C progressively loses factor VIII activity (Refs. 5, 6, and 30) with a loss at 24 hours approaching 50 percent of the initial factor VIII activity. However, if the plasma has been frozen solid within 6 hours of collection, the loss is only 10 percent (Ref. 5). Further fractionation of this plasma showed recovery of factor VIII in the cryoprecipitate is also 10 percent less both in vitro and in vivo. This loss appears to be acceptable to provide flexibility in processing blood from mobile blood collection facilities. Alternatively, mobile laboratories for cryoprecipitate production have been shown to be feasible (Ref. 31). As expected, outdated 21-day-old blood has been shown to be an unacceptable source of factor VIII because of the low starting levels of factor VIII (30 percent), lower cryoprecipitability of this material, and high plasma hemoglobin levels in blood more than 14 days old (Ref. 32).

c. *Plasma processing*—(1) *Centrifugation.* Centrifugation at 4,500 X g for 10 minutes leaves an average residual platelet count of 25,000/microliter (Ref. 5). Longer periods of centrifugation to reduce the level of platelets was not associated with any difference in factor VIII recovery in cryoprecipitate (Refs. 5 and 17).

(2) *Freezing rate, freezing temperature and volume of plasma.* Fifteen to thirty percent of the original factor VIII activity has been reported to be lost consistently during plasma freezing at −30 °C or −70 °C (Refs. 3, 13, 18, and 33) with subsequent storage at −30 °C. Later studies by Perkins (Ref. 19) and Rappaport (Ref. 34) have documented only a 10-percent loss in factor VIII activity after 2 months of plasma storage

and 20 percent after 1 year at −30 °C. They, as well as others (Ref. 35), have suggested that the major loss of factor VIII activity occurs with freezing and that factor VIII is stable during storage.

Factor VIII yield in frozen plasma can be improved by reducing the plasma volume (Refs. 5 and 13) or conversely, by increasing the size of the bag and thus the surface-to-volume ratio (Ref. 6). Therefore, studies of factor VIII recovery in plasma and its stability with storage must be done using the volume and bag size routinely used in blood banking. Certainly, some of the differences in factor VIII yield in the reported studies may be related to freezing rate if too many bags are put in the freezing container at one time thereby changing the time-temperature relationship and subsequently the rate of freezing. Although the freezing temperature of −30 °C vs. −70 °C has been suggested to be unimportant (Ref. 6), it is not clear that these experiments were done with the usually accepted volume of plasma, i.e., 250 mL. Other investigators have clearly shown that with full plasma volumes the freezing temperature must be −70 °C or below and that this temperature maintains 100 percent factor VIII recovery in the plasma for storage periods up to 3 months (Ref. 5).

Clearly, one significant technical problem may be the speed with which the factor VIII assay is done after sample thawing, as Hawkey (Ref. 18) has shown that after 1 hour at room temperature, the factor VIII level falls by 25 to 56 percent. In addition, Perkins (Ref. 19) has shown the instability of previously frozen factor VIII by documenting that refreezing plasma causes a further 50-percent reduction in factor VIII activity.

These data suggest that freezing at temperatures below −70 °C preserves 100 percent of factor VIII activity and subsequent storage at or below −30 °C will be associated with negligible loss of factor VIII activity. If freezing is done in a liquid medium, an overwrap should be used to prevent interaction of the freezing liquid with the plastic bag and its contents.

(3) *Thawing*—(a) *Temperature of thawing.* The most critical variables in cryoprecipitate production are probably the thawing temperature, the degree of thawing, i.e., to slush with ice crystals or completely thawed, and the solubility of cryoprecipitated factor VIII in its supernatant plasma. The usual yield of factor VIII in cryoprecipitate based on the factor VIII in fresh plasma has been in the range of 35 to 55 percent (Refs. 3, 13, 17, 35, and 36) with an average of 45 percent. These results are found when plasma is thawed at 4 °C in air, a process requiring 18 to 24 hours. Brown (Ref. 9) first demonstrated that rapid thawing of plasma at 6 to 8 °C, which takes approximately 90 minutes, yielded 87 percent of the original factor VIII content of the plasma minus the amount of factor VIII found in the cryo-poor supernatant which averaged 22 percent. However, in this study the plasma was transferred to 610 cc bags for freezing between metal plates, and therefore the results of this study are not applicable to clinical practice. Waumens (Ref. 12) reduplicated the original study, except he employed commonly used plasma volumes and bag sizes. There was a 26-percent factor VIII recovery in cryoprecipitate when plasma was thawed at 4 °C in air compared to 50 percent recovery for 6 to 8 °C water-thawed cryoprecipitate in this study. Subsequent in vivo recovery of this rapidly thawed cryoprecipitate was 90 percent of predicted. A subsequent study comparing 4 °C air to 4 °C water thawing has shown a consistent yield of 58 percent when water thawing is used compared to 45 percent when thawing was in air ($p < 0.01$) (Ref. 5). After in vivo infusion of these materials there was an even greater benefit of the water-thawed compared to the air-thawed material. However, these studies have not been confirmed by others (Refs. 6, 30, 37, and 38).

(b) *Partial thawing.* Cooke (Ref. 39) suggested that cryoprecipitates be thawed until the formation of ice crystals (slush phase) and that the crystals could act as a filter to retain the cryoprecipitate when the supernatant plasma was expressed. Both Burka (Ref. 17) and Slichter (Ref. 5) showed an increased recovery of factor VIII in cryoprecipitates brought to the slush phase compared to complete thawing at 4 °C in air—51 percent vs. 43 percent and 63 percent vs. 45 percent, respectively. However, in vivo infusions demonstrated that recovery was the same for both products (Ref. 5). It is difficult to standardize the end point at the slush phase.

(c) *Complete thawing.* The stability of cryoprecipitated factor VIII in superantant plasma after complete thawing has been studied. With 4 °C water thawing, cryoprecipitated factor VIII is stable for at least 2 hours (Refs. 5 and 6) with levels dropping to 61 percent plus or minus 12 at 33 hours ($p < 0.05$) and to 58 percent plus or minus 11 ($p < 0.01$) at 5 hours compared to a level of 69 percent plus or minus 11 for cryo in which the supernatant is immediately removed (Ref. 6). Similarly with air-thawed material at 4 °C, factor VIII activity in the cryoprecipitate decreases progressively after 12 hours of thawing with a concomitant increase in activity in the cryo supernatant plasma (Ref. 38).

After complete air-thaw factor VIII is stable for at least 6 hours in one study, 46 percent vs. 40 percent (Ref. 5), but significantly different at 4 hours in another, 53 percent vs. 47 percent ($p < 0.01$) (Ref. 6.).

(4) *Removal of supernatant plasma.* It does not seem to matter whether the cryo-poor supernatant plasma is drained off or expressed (Ref. 38).

5. *Cryoprecipitate Antihemophilic Factor storage and preparation for use*—a. *Freezing.* The available data indicate that for best results, cryoprecipitate after preparation should be refrozen at −30 °C or below (Ref. 6).

b. *Storage.* Factor VIII in frozen cryoprecipitate is stable at storage temperatures of −18 °C or below (Ref. 40).

c. *Thawing.* All studies reported in the literature have thawed the cryoprecipitate at 37 °C in a waterbath with gentle mixing. It is important that the bags are thawed for at least 15 minutes to ensure maximum factor VIII recovery (Ref. 5).

d. *Resuspension.* Cryoprecipitate has been reconstituted in either plasma, saline, or buffered saline (Ref. 28). When cryoprecipitate was prepared from small volumes of plasma (50 mL), thawed and resuspended, the dissolving solution made no difference (Ref. 28). Later studies have provided conflicting data on the best way to dissolve the cryoprecipitate. One study (Ref. 6) has shown that 20 ml. of plasma vs. 20 mL of saline gave factor VIII recoveries in the cryoprecipitate of 53 percent plus or minus 17 vs. 49 percent plus or minus 19, not statistically different. Furthermore, the duration of time that the material was within the bag before withdrawing did not affect factor VIII recovery. Another study (Ref. 5) has shown an optimum volume of 20 mL of saline left in the bag for 15 to 20 minutes before withdrawing gave factor VIII yields of 56 percent vs. 42 percent for 10 mL of plasma. These differences, confirmed in vivo by cryoprecipitate infusions into hemophiliac recipients, were significant ($p < 0.001$). In another study, draining the bag completely vs. leaving 5 mL of plasma made a significant difference in factor VIII recovery in vitro, 55 percent vs. 67 percent, respectively (Ref. 38).

Since saline resuspended cryoprecipitates can be provided on a nonblood group specific basis to most recipients, saline would seem to be the preferred diluent. If plasma is used as the diluent, the cryopercipitate has to be given as blood group ABO compatible.

How long the factor VIII activity is stable in the resuspended material has not been documented, but it has been shown that it cannot be refrozen or refrigerated without loss of 20 to 40 percent of the activity (Ref. 12).

e. *Pooling.* Pooling can be accomplished by syringe or vacuum devices, but should be done by experienced personnel either at the blood center before the material is sent out or at the hospital to ensure complete removal of all the concentrated material from each bag. When used for home care by hemophiliacs, appropriate training in its use must be provided. The pooled cryoprecipitates should be used as soon as possible, but must be used within 4 hours of entering the container. The resuspended cryoprecipitate should be left at room temperature prior to use (Ref. 12).

6. *Safety*—a. *Hepatitis.* Cryoprecipitate is expected to have the same risk of hepatitis as single donor plasma, but no data are available on this point. At least four cases of hepatitis have apparently been transmitted by cryoprecipitates (Refs. 43 through 46).

b. *Reactions.* Aherns (Ref. 47) reported 7 of 46 patients who had received cryoprecipitate from 4,363 donors had febrile reactions without other symptoms. Meyer (Ref. 43) reported no side-effects after the transfusion of 650 units of cryoprecipitate to 13 recipients.

Allergic reactions associated with cryoprecipitate are less severe and less frequent than with plasma (Ref. 40). Anaphylactic reactions have been described (Ref. 47).

c. *Factor VIII anticoagulants.* Two cases of patients with factor VIII inhibitors have specifically been reported following the administration of cryoprecipitates. One patient had received numerous plasma transfusions before being treated with cryoprecipitates for 13 days (Ref. 48) and the other had undergone three short courses of cryoprecipitate treatment in 4 weeks (Ref. 9). In 1969, 4 years after the introduction of cryoprecipitate, Strauss reported the incidence of acquired circulating anticoagulants in hemophilia A as 12 percent in the hemophilic patients studied (Ref. 49). However, the proportion was 16 of 77 (21 percent) of patients with factor VIII levels less than 1 percent of normal. Inhibitors did not occur on a familial basis, were found at a median age of 8 years (range 1 to 19 years), and developed after 20 to 90 exposure days to factor VIII. No data were given on the relationship between a specific transfusion product and inhibitor formation.

It is apparent that cryoprecipitate transfusions are associated with the development of inhibitors but there is no evidence that their frequency is either more or less than that associated with other transfusion products containing factor VIII.

d. *Hemolysis due to blood group antibodies.* The titers of anti-A and anti-B in cryoprecipitates are the same as those of the starting plasma (Refs. 40 through 42). Severe hemolytic transfusion reactions have been reported after the transfusion of cryoprecipitates dissolved in plasma to ABO incompatible recipients (Refs. 41 and 50).

e. *Blood group isoantigen content of cryoprecipitate.* McShine (Ref. 51) has documented an increase in immune anti-A and anti-B titers following the transfusion of cryoprecipitates to patients with hemophilia and von Willebrand's disease. The exact interpretation of this observation is not clear.

f. *Hyperfibrinogenemia.* Since fibrinogen is concentrated in cryoprecipitates, repeated infusions may lead to high levels of fibrinogen. This hyperfibrinogenemia is usually innocuous (Ref. 39). However, roulex formation may be seen in blood films and samples (Refs. 35 and 52).

g. *Pulmonary reaction.* One case report has documented pulmonary infiltrates and hypoxemia resulting from cryoprecipitate administration (Ref. 53).

7. *Effectiveness*—a. *Factor VIII deficiency (Hemophilia A).* It has been well documented that Cryoprecipitated Antihemophilic Factor (Human) is effective in controlling spontaneous and operative bleeding in hemophilia (Refs. 9, 13, 39, 41, and 54 through 58). The dosage can be calculated on the basis of recovery of factor VIII in cryoprecipitate and the plasma volume of the recipient. There is an initial rapid disappearance of factor VIII activity, with a half-life of 3 to 5 hours and a subsequent slower disappearance with an apparent half-life of 8 to 12 hours. Transfusions of factor VIII are usually given on a 12-hour schedule in an attempt to maintain minimum levels of 10- to 15-percent factor VIII activity in the postoperative patient to prevent bleeding (Ref. 58).

Cryoprecipitate has also been shown to be effective in preventing spontaneous bleeding in hemophilics (Refs. 59 and 60).

b. *von Willebrand's disease.* Perkins and Bennett (Refs. 61 and 62) have reported correction of the bleeding time defect and elevation of factor VIII levels in patients with von Willebrand's disease given cryoprecipitates. In two of Perkins patients, purified preparations of factor VIII (Hyland's Hemophil™ and the American Red Cross Intermediate Potency AHG) were unable to correct the bleeding time although the factor VIII level was increased. Subsequently, Profilate (Abbott Laboratories) was shown to have similar properties. (Ref. 63). In fact, several studies have shown that failure to correct the bleeding time with commercial AHG concentrates was associated with continued bleeding even though factor VIII activity, factor VIII antigen and VW factor associated with platelet aggregation to ristocetin were appropriately increased following transfusion (Refs. 63 through 65). In the same patients in these studies cryoprecipitate improved the bleeding time and controlled the bleeding.

c. *Hypofibrinogenemia.* Since cryoprecipitates contain approximately one-third to one-fifth of the available plasma fibrinogen, it is possible to use pooled cryoprecipitates as a source of fibrinogen (Refs. 3, 9, 12, 29, and 43). Presumably a small pool of cryoprecipitates would result in a decreased incidence of hepatitis compared to transfusion of fibrinogen pooled commercial derived from plasma (Ref. 66). A fibrinogen level of 60 mg/100 mL is probably adequate to provide hemostasis; a rise in fibrinogen of this amount was obtained in two hemophilic patients with the transfusion of 10 units of cryoprecipitate once a week (Ref. 68). A normal in vivo disappearance rate of fibrinogen transfused from this material has been documented in rabbits (Ref. 67). In addition, Hattersley (Ref. 69) described the successful management of surgery in a woman with congenital fibrinogenemia with cryoprecipitates, as well as the treatment of patients with disseminated intravascular coagulation (DIC) and hypofibrinogenemia (Ref. 70).

8. *Recommendations.* It is recommended that Cryoprecipitated Antihemophilic Factor (Human) be placed in Category I. Further recommendations include:

a. Standardization of the factor VIII assay should be done by each registered establishment with the United States Standard as a reference or alternatively with a donor plasma pool of 10 to 30 normal individuals. In addition, in vitro assay results of cryoprecipitates must be correlated with the results of in vivo cryoprecipitate transfusions in which factor VIII recovery and survival in hemophilia A recipients is measured to document further the validity of the assay. Significant changes in the production process must be validated with repeat in vitro and in vivo factor VIII assays.

b. Assays of at least four units of cryoprecipitate per month should be done by each registered establishment to assure maintainance of an average potency of no less than 80 units of factor VIII per bag.

c. The following studies should be done in the future by the general medical research community of Federal agencies:

(1) To define the freezing and storage temperature of plasma to provide optimal initial and long-term stability of factor VIII,

(2) To document the best freezing temperature of the cryoprecipitate and its stability at varying storage temperatures, i.e., −20 °C, −30 °C vs. −70 °C and

(3) To evaluatte the best way to dissolve the thawed cryoprecipitate to maximize factor VIII yield and stability after resuspension. Measurements should be made at room temperature.

d. Labeling should indicate that:

(1) Cryoprecipitate contains approximately 80 units of factor VIII/bag.

(2) Cryoprecipitate usually contains at least 150 mg of fibrinogen/bag.

(3) Good patient management requires monitoring treatment responses to cryoprecipitate transfusions with periodic plasma factor VIII or fibrinogen assays in hemophilia A and hypofibrinogenemic recipients, respectively.

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9. Single Donor Plasma (Human)

Generic Statement

1. *Introduction.* As indicated in the review of whole blood, an important consideration in the management of hemorrhage is maintenance of the circulating blood volume. During World War II, plasma was used extensively in combat settings for the treatment of acute blood loss (Ref. 1). Plasma was effective in improving the pathophysiologic defect resulting from trauma and acute blood loss, but there was a significant incidence of hepatitis in patients transfused with plasma. The product used at that time was prepared by pooling plasma from many individuals and freeze-drying large batches. It was noted that the incidence of hepatitis in patients transfused with plasma from a large pool of donors was greater than in patients transfused with plasma from a small pool of donors (Ref. 2). It has been shown that plasma retains hepatitis infectivity even when very dilute (Ref. 3).

At one time, it was believed that the risk of transmission of hepatitis by pooled donor plasma could be reduced by storing the plasma at 30 to 32 °C for 6 months or more prior to use (Ref. 4). In a well-controlled study, however, it was demonstrated that storage of plasma at 30 to 32 °C did not significantly reduce the risk of hepatitis (Ref. 5). In a summary of the clinical use of pooled plasma, the Committee on Plasma and Plasma Substitutes of the National Research Council concluded that the use of whole, pooled human plasma should be discouraged or discontinued unless a clear-cut case could be made that is met a unique requirement (Ref. 6). When plasma infusion is indicated, it is generally believed that the risk of causing post-infusion hepatitis can be greatly reduced by using Single Donor Plasma. Although a risk is still present, it is of the same order of magnitude as that associated with the transfusion of whole blood (Ref. 7).

Plasma is relatively stable during short term storage in the liquid state except for certain labile components. The factors affecting blood during short term storage in plastic bags have been considered in the review of stored Red Blood Cell Products. During prolonged storage there is apparently a loss of fluid from the contents of the plastic container and unless additional glucose is provided, fibrinogen will precipitate. In order to avoid the changes associated with storage in the liquid state, plasma should be stored in the frozen state. Once converted to the frozen state, little or no further change occurs in plasma

during long periods of storage. Individual units of plasma stored in this way, when thawed, are reasonably equivalent to the plasma at the time of freezing.

In addition to its volume and colloid osmotic effect, plasma has been used as a source of plasma coagulation factors. Two of the clotting factors (V and VIII) deteriorate rather rapidly in blood or plasma stored at 4 to 6 °C, but are relatively stable when stored in the frozen state (Refs. 8 through 10). Plasma separated from whole blood and rapidly frozen within 6 hours of collection contains almost all the original activity of factor V and factor VIII. This product is called "Single Donor Plasma-Fresh Frozen" to distinguish it from "Single Donor Plasma" that has been separated from whole blood after longer periods of storage at 4 to 6 °C. The other clotting factors are more stable in plasma, and there is little loss of activity of these clotting factors in plasma which is stored in the frozen state (Ref. 11). No doubt there are a number of plasma constituents that do deteriorate during storage, but the importance of these in transfusion therapy has not yet been demonstrated.

The blood group antigens, except those of the Lewis system, are related to the red blood cells and are not present in the plasma. Provided the blood cells are not inadvertently present in plasma, many of the risks related to transfusion of whole blood are not applicable to the use of plasma. However, there are risks related to antibodies that may be present in plasma. Plasma from individuals whose red blood cells lack A or B antigens contain antibodies active against the antigens which are lacking. Thus, anti-A and anti-B antibodies are present in the plasma of group O individuals; anti-A is present in the plasma of group B individuals, and anti-B is present in the plasma of group A individuals. Antibodies of this type have produced hemolytic transfusion reactions when transfused into individuals whose red blood cells contain the antigen for which the antibodies are specific (Refs. 12 through 14). It has been estimated that approximately 40 percent of group O individuals have sufficient antibody levels to cause hemolysis when their plasma is transfused into a group A recipient (Ref. 12). Hemolytic reactions have been reported with transfusion of plasma with anti-A and anti-B antibodies reactive in the cold (Refs. 12 and 13) as well as those reactive at 37 °C (Ref. 14). Antibodies reactive with other blood group antigens are much less likely to cause hemolysis when infused into a recipient whose red cells carry the relevant antigen (Ref. 15).

The development of vasoactive substances during plasma storage has been considered in the review of Stored Red Blood Cell Products. Mollison (Ref. 16) cites several reports indicating the occurrence of vasoactive substances apparently related to enzyme action on plasma proteins. In view of the recent observations on prekallikrein activator in plasma fractions (Ref. 17) it is clear that under certain circumstances vasoactive substances in plasma can be clinically significant.

2. *Indications for administration of Single Donor Plasma*—a. *Plasma volume expansion*—(1) *Effectiveness.* There is a considerable amount of clinical experience to indicate that infusion of plasma will effectively expand the plasma volume of the recipient, although there is a report that some hypovolemic recipients of homologous plasma may exhibit little expansion of plasma volume (Ref. 18). There are other products that do this equally well or better, but plasma is usually effective.

(2) *Safety.* (a) There is a risk of transmission of hepatitis with the infusion of plasma. This can be decreased by careful selection of donors and testing each unit for HBsAG, but a risk remains.

(b) There is a risk of hemolytic transfusion reaction associated with infusion of plasma without consideration of the ABO groups of the donor and recipient. This can be minimized by using group-specific plasma.

(3) *Benefit/risk.* There are several other products that produce the same or a similar clinical effect that are not associated with the same risks. In comparison to these products, the risks of using single donor plasma exceed the benefits for this indication.

b. *Supply of coagulation factors other than V and VIII*—(1) *Effectiveness.* Plasma clotting factors other than factors V and VIII are present in Single Donor Plasma in approximately normal amount. When administered intravenously, the activity in the circulation is proportional to the volume given. In severely deficient subjects, the circulating level can be increased to approximately 20 percent of normal without producing circulatory overload. This will often provide adequate hemostasis in uncomplicated cases. Such treatment is not adequate for severe bleeding episodes or when surgery is required.

(2) *Safety.* (a) The risk of hepatitis is present.

(b) The danger of circulatory overload must be considered when attempting to produced and maintain hemostatic clotting factor levels.

(3) *Benefit/risk.* When a relatively small increase in circulating levels of deficient coagulation factors is desired, the benefits of Single Donor Plasma are considerably greater than the associated risks for this indication.

c. *Recommendations.* (1) Single Donor Plasma (Human) should be placed in Category I.

(2) The use of this product for plasma fractionation should be encouraged.

(3) The name of the product should be "Single Donor Plasma".

(4) Indications for use in hypovolemia should be stated in labeling in relation to the hepatitis risk.

3. *Indications for the administration of Single Donor Plasma (Human)-Fresh Frozen*—a. *Source of factor V and VIII*—(1) *Effectiveness.* Plasma clotting factors, including factors V, are present in approximately normal amounts in this product. When administered intravenously, the circulating levels are increased proportionally to the volume infused. Levels approaching 20 percent of normal can be achieved by infusing this product into severely deficient patients.

(2) *Safety.* (a) The risk of hepatitis is present.

(b) The risk of circulatory overload is present.

(3) *Benefit/risk.* (a) This product is the only available source of factor V for human use. When used appropriately in patients with a bleeding episode associated with severe factor V deficiency, the benefits far exceed the risks.

(b) This product is a source of factor VIII in relatively small amounts. In view of the availability of other more concentrated sources of factor VIII the benefits expected are minimal.

(c) As a source of coagulation factors other than factor V and VIII, the benefits/risks are equivalent to those of Single Donor Plasma.

(4) *Recommendations.* (a) Single Donor Plasma (Human)-Fresh Frozen should be placed in Category I as a source for the administration of factor V.

(b) The name of the product should be "Single Donor Plasma (Human)-Fresh Frozen".

(c) The utilizaton of more of this product as a starting material for preparation of factor VIII concentrates should be encouraged.

(d) Studies to develop a factor V concentrate should be encouraged.

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10. Normal Serum Albumin (Human)

Generic Statement

1. *Introduction.* Albumin has at least two major functions, providing plasma-specific osmotic activity, and performing an important carrier function by transporting various substances in a bound state. Both of these functions relate to albumin in the plasma. Most albumin normally exists in extravascular sites. It is not known whether this albumin performs any specific functions or represents preformed, stored material available in case of sudden need.

a. *Plasma-specific osmotic activity.* Starling's law of fluid exchange across the capillary bed postulates a major role for plasma-specific osmotic activity in determining the distribution of fluid across the capillary membrane (Ref. 1). Normally, 70 percent of this activity is provided by albumin. Starling's law is not fundamentally a biological phenomenon, but is rather the application of basic and well-established physical principles to the biological situation. Some relatively recent "criticism" of this law (Ref. 2) has tended to confuse the validity of the formulation with the definition of the conditions under which it operates. Starling's original formulation was clearly a gross oversimplification, but was based on what was known at that time. Almost all relevant conditions have since been defined in much greater detail. Perhaps most importantly, it is now apparent that the conditions under which this principle operates are different in different parts of the body: Interstitial fluid pressure, interstitial albumin concentration, intravascular pressure, and even vascular permeability all vary in different organs and regions. Failure to take these differences into account explains most if not all of the discrepancies that have been claimed to exist relative to Starling's law (Ref. 3).

Understanding of the situtation is further complicated by some of the self-correcting features of lowering the concentration of albumin in the plasma. There is a concurrent lowering of the interstitial concentration of albumin, minimizing the change in the transcapillary gradient, a rise in interstitial fluid pressure as fluid accumulates which also resists egress of fluid from the capillaries, and, in many organs, an increase in lymphatic return to minimize the extravascular accumulation of edema. In addition, the normal level of albumin in plasma is well above that necessary to prevent gross extravascular shifts of fluid. Nevertheless, numerous studies have shown that when albumin concentration is lowered, it is easier to induce the formation of interstitial edema, and this is the essence of Starling's formulation. Clinical experience is also concordant with the above relationship, but the quantitative details are far from clear. Certain populations of patients can tolerate hemodilution quite well; the level of hypo-albuminemia at which clinically worrisome changes appear in other patients is very variable. Thus, empiricism and experience still dominate clinical practice, and much more detailed knowledge of transcapillary fluid dynamic will be needed before clinical use of albumin for its osmotic effects can be placed on a firm rational basis. Some of what is known about various disease states will be reviewed later.

b. *Carrier protein.* Albumin is second only to hemoglobin as the major "carrier" material in the blood. A large number of naturally occurring and therapeutically administered substances are transported in the blood partially bound to albumin (Ref. 4). Albumin can bind substances with positive or negative charges, as well as a wide variety of uncharged substances. Among the natural substances carried by albumin, the most important may be the long-chain fatty acids. These are the major fuel substrates for the body, but are toxic in even small concentrations if unbound or nonesterified. They are carried almost totally bound to albumin, with varying degrees of affinity (Ref. 5). In the bound form they do not damage cell membranes or capillary walls. A variety of other lipids are also carried largely bound to albumin, as are certain divalent metals (calcium, copper and possibly zinc), bilirubin, tryptophan which is the only amino acid present in bound form in the circulation, and many more substances. The binding of bilirubin as in hemolytic disease of the newborn and of tryptophan as in cases of hepatic encephalopathy may be closely related to these and other

disease states. Similar clinical disorders have not been identified for the bound lipids, although marked hyperlipemia is characteristic of severe hypo-albuminemia and reverses when albumin is administered. Binding of certain hormones may also have physiologic significance, as may the binding and transport of many other substances normally carried in the blood.

A very large variety of drugs are carried in the blood partially bound to albumin (Ref. 6). The important pharmacodynamic features of these drugs such as levels in the interstitial fluid and urinary excretion are usually determined by the proportion of the drug that is unbound.

In spite of these considerations, it has been difficult to attribute specific harmful effects to loss of the carrier function of albumin in severe hypoalbuminemia except in hemolytic disease of the newborn. There are many interrelationships between and among substances bound to albumin with some, especially the fatty acids and certain drugs, interfering with the binding of many others. Competitive binding must certainly accentuate effects at lower-than-normal concentrations of albumin. As knowledge of pharmacodynamics increases, the binding function of albumin is likely to be increasingly recognized as important.

c. *Normal albumin dynamics.* Albumin has been difficult to study because of its long persistence, 15 to 20 days, and multicompartmental distribution with varying equilibration times. This requires a long period of steady state conditions for the establishment of equilibration curves and precludes the study of many important but unstable clinical states. The normal total mass of albumin in a 70 kg adult probably exceeds 300 grams with a turnover of 15 grams per day (Refs. 4 and 7).

(1) *Albumin production.* Albumin is synthesized in the liver and seems to be released directly into hepatic sinusoidal blood rather than by way of the thoracic duct. The rate of synthesis is very sensitive to the supply of amino acids presented to the liver. Complete fasting can reduce the rate of synthesis by 50 percent within a few hours, with recovery beginning within minutes of refeeding. A number of amino acids are specifically effective in stimulating synthesis of albumin. All of these also stimulate synthesis of urea. Some of these, tryptophan and ornithine specifically, are incorporated sparingly or not all all into the protein synthesized.

Hormonal balance probably influences the rates of synthesis for albumin, Cortisol, thyroxine, and growth hormone appear to stimulate synthesis. Although not specifically studied, glucagon is likely to have the opposite effect. A certain amount of insulin may be necessary to achieve maximum rates of albumin synthesis.

One of the main mechanisms controlling albumin metoliusm is the concentration of colloid in the interstitial spaces of the liver (Ref. 8). As this concentration increases, the rate of synthesis of albumin decreases. Almost any osmotically active material seems to be effective in decreasing the rate of synthesis: dextran, gamma globulin, or albumin itself. Some authors seem to believe that hyperosmolality due even to small molecular species will inhibit synthesis. The rate of degradation does not decrease proportionately, and the level of albumin in the blood falls until the concentration of large molecular colloid in the hepatic interstitial space returns to normal.

(2) *Degradation of albumin.* Remarkably little is known about the site or control of degradation of albumin, when one considers its physiological importance and the large amount, about 15 grams, normally catabolized daily. Several experiments suggest that synthesis and degradation are not interdependent. In the absence of better data, the liver seems to be a likely site for degradation of albumin, but in vitro studies on liver slices do not confirm this.

Rates of degradation increase markedly with induced hyperalbuminemia and decrease during starvation. The great void in our knowledge regarding albumin degradation and its control seriously impair understanding of the overall control of albumin metabolism.

(3) *Body distribution of albumin.* Although there is some disagreement about details, two different methods of analysis indicate that most of the albumin is normally outside the plasma, probably 60 to 67 percent (Ref. 9). Most of the extravascular albumin is present in the skin, less in muscle which has a low concentration of albumin in a large total volume of interstitial fluid, less in the gut, and very little in the liver or other viscera. Extravascular albumin equilibrates at different rates with the plasma pool; it is most rapid for muscle, slower for the skin, and very slow for the gut. The extravascular albumin returns to the plasma mainly by the thoracic duct. The rate of return of albumin from all extravascular sites is very much slower than the rate of return of interstitial water. This suggests that there is a distinct holding mechanism for albumin in the interstitial matrix similar to molecular sieving, with different features of the general process in different tissues.

2. *Indications for the clinical use of albumin.* Albumin is used clinically for many different reasons. The survey recently reported by Sgouris and Dorsey generally confirms the usually stated categories of reasons given for usage; these are the only such data available in the U. S. (Ref. 10). Some of the present albumin usage is plainly mistaken because of oversights in ordering its administration (Ref. 11), but much of its use follows a certain logic. Before considering some of these specific categories, some general comments may be useful.

Personal experience of members of the Panel indicates that very few physicians, including well-trained house staff, understand the arithmetic of the distribution of administered albumin. Many physicians are completely unaware of the existence of extravascular stores of this protein in equilibrium with the plasma; very few know the approximate size of these stores or are aware of the complex, changing relationships between blood levels and interstitial levels that occur as hypoalbuminemia develops. All of these voids in knowledge lead to very gross underestimations of the amount of albumin needed to achieve a desired effect. Most clinicians also seriously underestimate the half-life of human albumin, but this error is not enough to offset their gross underestimation of requirements. Just as importantly, few clinicians can really explain why the blood level of albumin they have chosen is desirable. Perhaps the most rational practitioners are the complete empiricists who administer albumin until the blood levels increase or, if appropriate, blood pressure, urinary output or arterial oxygen pressure increase, or until edema subsides. Sometimes the end point of this pattern of administration is very vague, but usually it is a rational goal. Given the regrettable ignorance of most clinicians about the physiology of the product they are prescribing and its quantitative aspects, the empirical approach is perhaps the safest for the individual patient. It is not the best for the blood, plasma, and albumin supply system, however. As Lundsgaard-Hansen has implied for Switzerland (Ref. 12), and also in the United States, albumin is used in too many patients in quantities too small to do many of them much good even if they did need it.

a. *Hypovolemia*—(1) *Rationale and effectiveness for use in hypovolemia.* Probably the commonest reason for using albumin is to expand blood volume. Occasionally it is used as the primary agent for this purpose; more commonly it is used to supplement simple salt solutions, and occasionally it is used to supplement administration of sedimented or frozen, washed red cells. Some of this use is for a combined indication of hypovolemia with hypoalbuminemia when an increase in cardiac output is sought in such a patient who may also already have peripheral edema.

The normal response to hemorrhage consists of an initial intravascular shift of protein-poor interstitial fluid. There is a gradual shift of albumin back into the vascular space, largely via the thoracic duct, with an overall "buffering" of the intravascular mass of albumin by a greater decrement in the extravascular mass once equilibrium is reached (Refs. 7 and 13). This is characteristic of the response to sudden loss of albumin regardless of the cause. The sites of extravascular albumin storage are primarily the very organs in which capillary pressure is reduced in response to hypovolemia, especially the skin. This suggests that physical forces have much to do with this buffering mechanism. No other control mechanism has been identified or suggested.

The rate of synthesis of albumin varies inversely, and the rate of degradation varies directly with its concentration in the interstitial space of the liver. The mechanisms of this regulation are unknown. The intravascular concentration of albumin is thus restored back nearly to normal within a few days even without supplementation by administered albumin.

One disadvantage to the use of albumin to restore blood volume is its long persistence. If the need for red cells becomes apparent after administration of a large volume of albumin, one must either wait for its equilibration throughout the system, or perform phlebotomy or plasmapheresis, or run the risk of overloading the circulation with the administered cells. The practical disadvantages of albumin therapy are those of cost and availability.

(2) *Burns.* Burn treatment represents a special situation. Pruitt and associates (Ref. 14) and Baxter (Ref. 59) have presented evidence in patients that supplemental albumin with isotonic or slightly hypotonic salt solutions contributes nothing to the expansion of the plasma volume in the first 24 hours after a major burn. Its use in these circumstances does not seem rational. A recent study, however, indicates that supplemental albumin may be useful in the early post-burn period if used with hypertonic saline solutions (Ref. 60). Interestingly, these patients had normal rates of disappearance of albumin from the circulation, in marked contrast to patients treated with other regimens and studied in this and other series. A number of unanswered questions remain, and more study is clearly needed.

Large amounts of protein are lost into the burn wound for significant periods of time. One of the major sites of albumin storage, i.e., the skin, is partially destroyed and the albumin that has been thermally altered by the burn is almost certainly removed at an accelerated rate. Paralytic ileus usually occurs in burned patients and interferes with nutrition. Some degree of infection is always present, and a prolonged maximum stress response with a prolonged catabolic state is also produced. Massive edema usually develops. Repeated skin grafting operations are necessary. All of this can create the need for administering very large amounts of albumin therapeutically. There is still disagreement about administering albumin early after burn injury, but even extensive nutritional support may have to be supplemented with the administration of albumin later in the care of such patients until the burn wounds are healed.

(3) *Miscellaneous uses of albumin in hypovolemia.* Albumin is used to replace blood volume in a variety of special situations, such as exchange plasmapheresis, "total body washout," and to supplement extensive use of red blood cell concentrates or frozen, washed red blood cells. In these circumstances, clotting factors are not replaced exogenously, and there is often a lack of awareness of the significant amount of citrate in these preparations. Other indications for the use of albumin are quite nonspecific and represent combinations of hypovolemia and hypoalbuminemia as in pancreatitis, trauma, hemorrhage, "shock," cardiopulmonary bypass, and hemodialysis.

(4) *Effectiveness of the use of albumin for hypovolemia.* The older literature confirms the effectiveness of albumin prepared by the Cohn fractionation method in supplementing circulating levels of albumin in the recipient with a variety of illnesses, including hypovolemia, and in restoring blood volume toward normal (Ref. 15). The specific treatment of blood loss with plasma proteins, however, has been an uncertain area. There is much experimental and some clinical evidence of less-than-expected effectiveness of albumin administration in promoting survival (Ref. 16). Most of these studies used raw plasma rather than albumin, and it is likely that there were differences in the degree of protein denaturation. The role of albumin prepared by Cohn fractionation in resuscitating patients from hemorrhage remains to be defined, especially as it can be compared with other currently used treatments for this condition. Recent evidence indicates that simple electrolyte solutions may be equally effective in many patients at far less cost (see section on acute pulmonary insufficiency).

(5) *Safety albumin for use in hypovolemia.* Albumin appears to be inherently safe. The main potential problems of its use relate to its physiologic effects. The restoration of blood volume in a hypovolemic patient may result in renewed bleeding from transected blood vessels. The expansion of blood volume by administering albumin without red blood cells was discussed above. Concentrated albumin, above 5 grams/dL, requires the administration of additional noncolloid containing fluids unless the patient is already overload with interstitial fluid. Administering to much albumin can overload the circulation and precipitate pulmonary edema.

(6) *Benefit/risk evaluation of albumin for use in hypovolemia.* When properly used for hemorrhage, albumin appears to be significantly beneficial at very little risk.

(7) *Recommendations for albumin administration in hypovolemia.* Hypovolemia is a valid indication for administering albumin. It is not however clear that albumin adds any therapeutic effect to the use of simpler electrolyte solutions when treating limited hemorrhages. (See the section on pulmonary insufficiency).

(8) *Labeling changes for use in hypovolemia.* The appropriate precautions discussed here should be included in all labeling. Use of 25-percent albumin as the primary treatment for hypovolemia is questionable, and it should not be used as such unless the patient is already edematous. If the 5-percent preparation of albumin is not available, 25 percent albumin may be used with concurrent administration of an appropriate volume and kind of fluid.

b. *Hypoproteinemia*—(1) *Rationale and effectiveness for use in hypoproteinemia.* Albumin is ofter used to mobilize or prevent the formation of

peripheral edema in patients with significant hypoalbuminemia. As previously discussed, the deficit in albumin is usually grossly underestimated, and in situations with large continuing losses of albumin this treatment will have very temporary effects. Aside from the specific effects on pulmonary function, it is not clear what is gained therapeutically by increasing the plasma concentration of albumin, and what, it any, is the minimum desirable level. Hypoproteinemia is one of the conditions that is associated with impaired healing of wounds (Red. 17). It is not known to what extent hypoalbuminemia contributes to this, and how effective the infusion of albumin might be in restoring the capacity for healing wounds. Congenital analbuminemia is rare but seemingly well tolerated. There are undoubtedly several compensatory mechanisms in this condition which may not operate in acquired hypoalbuminemia. To the extent that the cause of the hypoalbuminemia can be identified and corrected, it should be, as this will be much more effective from every viewpoint.

There are a number of clinical situations in which chronic hypoproteinemia is common. Each has special considerations. There are at least two separate problems in patients with cirrhosis. The rate of sythesis of albumin may be decreased because of functional impairment of the liver, but ofter this can be returned to normal by stopping the ingestion of alcohol and providing adequate nutrition (Ref. 18). The rate of degradation of albumin is also decreased, but not sufficiently to preserve normal blood levels. Administered albumin effectively, but temporarily, restores blood levels toward normal in the absence of ascites. If ascites is present, the sequestration of albumin in ascitic fluid can constitute a marked distributional abnormality. Albumin appears to enter the ascites directly from the liver. The total mass of body albumin may be greater than normal, but severe hypoalbuminemia is usually present. Administered albumin ofter has little effect in reducing the ascites if the ascites has proven resistant to currently available diuretics and/or aldosterone antagonists.

The nephortic syndrome epitomizes the pathological losses of albumin that can occur with renal disease. With marked albuminuria, synthetic rates are markedly increased provided nutrition is maintained. Rates of albumin degradation are decreased. The compensatory shift of extravascular albumin to the plasma can be altered by extensive peripheral edema with expansion of the interstitial space. Infusions of albumin can help mobilize edema, but he effects will be very brief as long as rapid loss of albumin continues.

Interpretation of albumin dynamics in the patient with gastrointestinal disease can be very difficult. The gut can certainly shed large quantities of albumin and, like the kidney, constitutes a site of major loss. Many gastrointestinal diseases will also produce impaired absorption of nutrients (Ref. 19). The responses in albumin production of these patients to total parenteral nutrition indicates that malabsorption dominates in many patients with inflammatory bowel disease.

Pemphigus is another condition in which very large amounts of albumin can be lost continously. If the oral cavity is involved, nutrition may also be impaired. Any extensive exfoliative condition of the skin can do the same.

(2) *Safety of the administration of albumin in hypoproteinemia.* Again, caution is required when using albumin in an anemic patient, because the expansion of plasma volume will decrease the concentration of hemoglobin in the blood.

(3) *Benefit/risk of the administration of albumin for hypoproteinemia.* Older literature and current clinical experience supports the effectiveness of currently available preparations of albumin in restoring the plasma levels of albumin back toward normal in these conditions, if the distributional aberrations are taken into consideration (Ref. 15). With rapid continued loss of albumin, the effects of its administration will be very transient.

The risk of administrating albumin is low except when concentrated albumin is given rapidly to an edematous, hypoalbuminemic patient. It is estimated that 1 gram of albumin holds 17 to 18 mL water in the plasma volume. Rapid administration of the 25-percent concentration material to an edematous patient can easily precipitate pulmonary edema. The concurrent administration of a non plasma expanding diuretic to reduce plasma volume may be useful in this situation, but careful monitoring and slower rates of administration are even more desirable.

(4) *Recommendations for the administration of albumin in hypoproteinemia.* More basic research is needed to define the levels at which hypoalbuminemia becomes harmful, and when correction by supplementation is worthwhile.

(5) *Recommendations for labeling of albumin for administration for hypoproteinemia.* Labeling should emphasize the danger of inducing pulmonary edema when giving concentrated (25 percent) albumin to an edematous, hypoalbuminemic patient. Correction of the underlying defect should also be emphasized as the preferable approach to treating hypoalbuminemia.

c. *Acute pulmonary insufficiency*—(1) *Rationale for use of albumin in acute pulmonary insufficiency.* There is considerable disagreement concerning the role of plasma concentrations in the development and treatment of acute pulmonary insufficiency. On one side, it is clear that decreasing the albumin concentration in the prefusate of the isolated lung increases the ease with which pulmonary edema forms (Refs. 20 and 21). Arterial oxygenation in patients with acute pulmonary insufficiency may improve with the concurrent administration of albumin and diuretics (Refs. 22 and 23). Some studies show a significant degree of hypoalbuminemia in most patients with acute respiratory failure not due to left ventricular failure (Refs. 23 through 25, 61 and 62), but this does not prove a cause-and-effect relationship. Skillman et al. have shown in a prospective, semi-controlled study on surgical patients that adding albumin to the therapeutic fluid regimen is associated with less subsequent impairment of oxygenation (Ref. 26), but the patients were treated with predetermined volumes of fluid. Elective hemodilution with solutions containing albumin appeared to cause less impairment of pulmonary function than hemodilution with simple salt solutions, but the studies were not concurrent or randomized (Ref. 63).

On the other side of the question, there are now several prospective, well-controlled studies in patients resuscitated from serious injury or studied through major intraabdominal operations, in which treatment with isosmotic albumin was compared with treatment with simple electolyte solutions. No differences were observed, either in gross clinical results or on sophisticated testing of pulmonary function (Refs. 64 and 65). Several studies are noteworthy because of the surprisingly small decreases in albumin concentration that were produced by the use of simple salt solutions as treatment for major hemorrhages (Refs. 64 and 66). In one study, serum colloid osmotic pressure was reduced to about 60 percent of normal, perhaps because the study group was comprised of older patients undergoing extensive

retroperitoneal dissection (Ref. 65). These patients were carried through the operative period with acceptable pulmonary function, at least equal to that of the control group treated with supplemental albumin. These patients were very carefully monitored for changes in pulmonary capillary wedge pressure, however, and this may be a requirement for such methods in patients of this type. More clinical studies of a similar type are currently underway, involving various specific groups of patients. Such studies will greatly clarify the extent to which supplemental albumin is needed (or not needed) in the treatment of acutely ill patients. The best current data relate to patients treated for acute hemorrhage and indicate that extensive blood loss can be treated with simple electrolyte solutions without use of supplemental albumin, even when red cell preparations must also be used.

The physiological basis for this surprising result is now being investigated in controlled laboratory experiments. Much of this work is also still in progress, but studies of the lymphatic drainage of the lungs indicates that the consentration of albumin in the interstitial fluid of the lungs falls rapidly with an acute decrease in the concentration in the plasma, thereby tending to minimize a change in the transcapillary gradient. In addition, the rate of pulmonary lymphatic drainage can increase markedly, protecting the lungs from flooding. One result of this relative resistance of the lungs to flooding is that excessive amounts of salt and water tend to be sequestered in other sites in the body, especially skin, fat, and muscle (Ref. 67).

Most of these studies only cover the acute phase and deal with otherwise normal individuals. These results might not occur in other clinical circumstances. It is worth noting that the incidence of pulmonary dysfunction after use of large amounts of salt and water may also be effected by post-resuscitative renal function, indicating that the compensatory mechanisms are limited (Refs. 68 and 69). Even with gross overloading with salt and water, hydrostatic pressure may be a more important determinant of the formation of pulmonary edema than plasma osmotic pressure (Refs. 65 and 70).

The proper use of supplemental albumin in patients with already established acute pulmonary insufficiency without chronic lung disease is even less clear. Several published studies indicate a rough correlation between the degree of hypoproteinemia and various measures of pulmonary insufficiency in such patients, but there is no study showing clear improvement following administration of albumin only (Refs. 23 through 25, 61 and 62). It is possible, especially in infected patients, that this concurrence of hypoproteinemia and pulmonary insufficiency represents the effects of another common cause rather than direct cause and effect. At least one study now in progress on such patients who are also hypovolemic compares resuscitation with simple salt solutions versus isomotic albumin (Ref. 71). No functional differences were found. It is unfortunate that concentrated albumin was not used as a test solution. As with resuscitation from simple blood loss, however, the expected benefits of supplemental albumin in hypoproteinemic patients in ventilatory failure are proving unexpectedly difficult to document.

Established pulmonary failure is often associated with markedly increased amounts of albumin in the lungs, reflecting a probable increase in pulmonary capillary premeability (Ref. 27). If such indeed, occurs, administering albumin would be of little help. Such lesions may be irregular in distribution, however, (Ref. 72), and the relatively normal areas of lung might be protected by maintaining albumin concentrations in the plasma near normal. Recent studies in large primates (Refs. 28 and 29) and in rodents (Ref. 30) indicate that use of albumin or plasma during resusitation from severe hemorrhage may in fact increase the sequestration of fluid in the lungs and the pulmonary ultrastructural changes demonstrable after resuscitation. Some studies in primates come to the opposite conclusion (Ref. 31). Such studies in animals must be balanced against the reported similar studies in patients, with one exception discussed below, in which use of albumin was not demonstrably harmful.

There is a single published study in which use of supplemental albumin in acutely injured patients was associated with higher morbidity, especially pulmonary insufficiency, than use of simple electrolyte solutions (Ref. 73). The patients treated with albumin in this study received the albumin on a predetermined schedule, and had demonstrably higher blood volumes and very much higher cardiac-filling pressures. It seems from the data that too much albumin was given to these patients, with overexpansion of blood volumes to the point of pulmonary edema.

(2) *Safety of albumin administration for acute pulmonary insufficiency.* As noted, the possibility exists that in certain situations, albumin might worsen problems of ventilation and gas exchange. Aside from overexpansion of blood volume, however, this is conjectural, and current evidence favors avoiding hypoalbuminemia if possible in patients in acute respiratory failure. The expected beneficial effects, however, still lack clear documentation in properly controlled studies.

(3) *Benefit/risk of administration of albumin for acute pulmonary insufficiency.* The benefits probably significantly outweigh the risks for this indication, but more study is clearly needed.

(4) *Recommendations for the administration of albumin in acute pulmonary insufficiency.* Considerably more investigation is urgently needed on this important problem. Some of this research is now underway.

(5) *Labeling recommendations for the administration in acute pulmonary insufficiency.* In the absence of better and more complete data, no firm recommendations can be made. Any labeling dealing with this indication should indicate that some controversy exists.

d. *Malnutrition*—(1) *Rationale and effectiveness of the administration of albumin in malnutrition.* Hypoalbuminemia develops during protein or protein-calorie deprivation. Inadequate nutritional intake leads to rapid diminution in rates of albumin synthesis and degradation with some decrease in the total mass of albumin and the expected shift of extra-vascular albumin into the vascular space. This compensatory shift which masks much of the true decrease in total body albumin mass had led to numerous statements about the poor correlation between serum levels of albumin and nutritional status. Better understanding of the dynamic processes involved and more precise methods of measurement have shown that the serum level of albumin does indeed vary in the expected manner with alterations in nutritional status. While administration of albumin is effective in temporarily correcting nutritional hypoalbuminemia (Ref. 15), the clearly preferred treatment is the correction of the malnutrition. Sometimes this requires the use of total parenteral nutrition, which carries with it some danger, so that costs and benefits must be carefully evaluated.

Albumin given intravenously is not a good sole source of high quality protein because of its relative deficiency in two essential amino acids, leucine and

tryptophan, and because of its slow rate of degradation after infusion. It can, however, support growth for short periods. Better products are now available consisting of mixtures of amino acids for parenteral use. These do not require human sources for production and are superior products for parenteral nutritional use.

(2) *Safety of the administration of albumin for malnutrition.* Albumin is safe, if less than optimally effective when used for this indication.

(3) *Benefit/risk of the administration of albumin for malnutrition.* While the benefit/risk ratio is acceptable because of the low risk, there are clearly more effective and efficient methods of treatment of malnutrition available.

(4) *Recommendation for the administration of albumin for malnutrition.* It is recommended that this indication for use be eliminated.

(5) *Labeling recommendations for the administration of albumin for malnutrition.* Labeling should not promote or suggest the use of albumin as either the primary or a supplemental treatment for nutritional deficiencies when preferable forms of treatment are available. Use of these other methods should be strongly suggested, if malnutrition is discussed at all in the labeling.

e. *Trauma and infection*—(1) *Rational for the effectiveness of the administration of albumin for trauma and infection.* Synthesis of albumin decreases markedly after injury in animals; only about half of this is due to inanition (Ref. 32). It is likely that the same occurs in man, as the degree of hypo-albuminemia that develops is greater than would be expected from sequestration in wounds. In some states of infection, hypergammaglobulinemia may be associated with a decrease in the synthesis of albumin, but the quantitative aspects of this relationship are unclear. In addition there can be sequestration of large amounts of albumin in areas of injury, operation, or infection.

Severe trauma and infection are both characterized by prolonged negative nitrogen balance (catabolism) far exceeding that due to starvation, a state that is resistant to supplemental nutrition. An expansion of the interstitial fluid space also occurs. It is likely that much of this is hormonally mediated, but the details of the involved mechanisms are not at all clear (Ref. 33). It seems likely that albumin shares in the generalized protein catabolism. However, the rapid changes in metabolism, pool sizes, and clinical condition have made detailed studies of the metabolism and distribution of albumin in such patients impossible. This is very unfortunate, as these patients represent perhaps the single largest group of potential recipients of albumin in current medical practice. The fate of albumin administered to these patient, for example, is largely unknown.

Supplemental albumin is effective in restoring blood levels after extensive operations or major injury; the effectiveness of albumin administration seems to be somewhat less with major infection. Whether this difference is due to greater sequestration or to increased catabolism is uncertain.

(2) *Safety of the administration of albumin for trauma and infection.* If overloading of the circulation can be avoided, the use of albumin seems safe for these indications.

(3) *Benefit/risk of the administration of albumin for trauma and infection.* With a very low risk, almost any benefit establishes an acceptable ratio. There probably is some benefit in these circumstances, although its exact nature and value is not clear.

(4) *Recommendations for the administration of albumin for trauma and infection.* Methods for evaluating rates of synthesis, rate of degradation, and pool sizes which measure the effects of albumin administered for these conditions are needed.

(5) *Labeling recommendations for the administration of albumin for trauma and infection.* There are no specific recommendations for labeling changes.

f. *Post-operative hypoproteinemia.* Operations involving large wounds are accompanied and followed by sequestration and loss of albumin (Ref. 34). The quantities involved can be very large, especially with extensive procedures in the pelvis or retroperitoneum, where effective immediate sealing of the wound is difficult. If drainage to the outside is present, significant losses may continue for days. These sudden losses occur in a setting of protein starvation, so that the anticipated increase in rates of synthesis is almost certainly delayed. Recent studies indicate that parenteral supplementation of nutrition with amino acids is sufficient to increase rates of synthesis of albumin significantly in post-operative patients (Ref. 35). With extensive surgical procedures, however, infusion of albumin itself during and after the operation may be necessary.

g. *Hemolytic disease of the newborn.* It is the unbound unconjugated fraction of plasma bilirubin that causes neurological damage in icteric newborns. Albumin is the major plasma binding substance and thus offers some protection against brain injury by bilirubin. Synthesis of albumin may be impaired in some sick newborns, and the concentration of the patient's albumin must be measured and maintained near normal. If exchange transfusion becomes necessary, supplemental albumin before and during the exchange may increase the efficiency of removal of bilirubin from infants, even those with relatively normal concentrations of albumin (Refs. 36 through 38). If care is taken to avoid circulatory overloading from induced hypervolemia, little harm and some benefit probably result. Drugs that compete with bilirubin for binding to albumin must be avoided in these infants. Pertinent specific references should be cited in the labeling if this indication is listed.

h. *Cardiopulmonary bypass*—(1) *Rational for the administration of albumin in cardiopulmonary bypass.* Albumin is often used to avoid induction of excessive edema during cardiopulmonary bypass with priming solutions which do not contain blood, and with massive single-pass exchange transfusions ("total body washout"). The amount of albumin that is used varies considerably, and it is clear that normal levels are not necessary during bypass to achieve good results. The minimum acceptable levels are also not known, but restoration of albumin concentration after bypass is clearly more important than maintenance during bypass.

(2) *Safety of the administration of albumin for cardiopulmonary bypass.* Under the circumstances of close monitoring during treatment that prevails in these patients, use of albumin to mobilize excess salt and water from the interstitial space seems safe.

(3) *Benefit/risk of the administration of albumin for cardiopulmonary bypass.* With proper monitoring to detect left ventricular failure, the benefit/risk ratio is acceptible.

(4) *Recommendations for the administration of albumin for cardiopulmonary bypass.* Clinical data indicating the most efficient time for administration and the lowest doses needed for acceptable effects would help reduce wastage of albumin.

(5) *Labeling recommendations for albumin administration for cardiopulmonary bypass.* Labeling should emphasize the need for careful monitoring of patients and the lack of data needed to define the most efficient patterns of administration of albumin if this indication is listed.

i. *Cerebral edema.* Cerebral edema has appeared briefly in the literature as an indication for the parenteral administration of human albumin (Refs.

39 through 41). The clinical experience cited is very brief, completely uncontrolled, and unsubstantiated by documentation of preexisting hypoalbuminemia that was corrected. Current concepts of cerebral edema focus on the mobilization of intracellular fluid as well, by use of small molecular, osmotically active substances such as mannitol. There is little theoretical, experimental, or clinical data to support the use of albumin for the treatment of cerebral edema in a nonhypoalbuminemic patient. This indication should not be approved for labeling.

j. *Miscellaneous.* Most other listed indications for use, such as pancreatitis and toxemia, are not specific but are rather diseases in which hypovolemia and/or hypoalbuminemia may occur in varying combinations of severity.

3. *Safety issues related to the manufacture of albumin*—a. *Polymers.* There is significant variation in the proportion of albumin which exists in polymeric form in various preparations. Factors which bear on this include the quality of the starting material, the amount and duration of heating, the temperature during storage, the method of fractionation, and the concentration of the albumin in solution (Refs. 42 through 47). It is noteworthy that the percentage of monomer declines with reheating during the manufacturing process, increasing age of the starting material, and prolongation of storage. Some manufacturers achieve a product with over 90 percent of the albumin in monomeric form; for others, some lots may be as low as 70 percent and perhaps even lower (Refs. 44 through 47). For all manufacturers, the monomeric fraction apparently decreases with storage, but apparently at different rates.

There is very little evidence on the effectiveness of polymeric albumin. What data there are suggest that it is removed rapidly from the circulation after it is infused. There seems to be only one study on the fate of infused human albumin dimers in man (Ref. 48). That study revealed only a modest shortening of the biological half-life of the albumin in the few subjects studied. There seems to be no evidence of albumin dimers dissociating back to monomers after infusion. If this is the case, the dimeric form would then have half the osmotic effect of the monomeric form on a weight basis. It is likely, but not proven, that higher polymers contribute little or nothing to the desired therapeutic effects of infused albumin. There is, therefore, concern about the variation in composition of albumin monomers and oligomers which may in fact result in surprisingly wide variations in effect in vivo. Clearly, much more data are necessary to evaluate the significance of oligomers in these products, but it seems prudent and reasonable to require better control of the amount of polymers in the products pending the availability of such data.

b. *Placental source materials.* A portion of the source albumin currently used in manufacturing by one producer is derived from human placentas. There are some differences in the processing and properties of this source material compared to venous plasma from blood or plasma donors.

Placental material from each donor is not individually tested for hepatitis B surface antigen (HBsAg). Data from the single current manufacturer indicates that at least 0.2 percent (and probably more) of their donor population carry detectable HBsAg. Numerous previous studies have shown that HBsAg can be carried through the usual cold ethanol fractionation process and be detectable in albumin (Refs. 49 through 53). Similar studies with the exact method used to process placental material have not been done. It is unlikely that albumin produced from placental sources is infectious for hepatitis B virus (HBV), but it is likely that some HBsAg is carried through processing into the final product. The concentration is likely to be too low to be detected by currently available methods, but it is not known if such low concentrations can produce a detectable antibody response in recipients of this product. If such occurs, it could certainly result in diagnostic confusion and uncertainty when later detected in former recipients. If such is to be expected with significant frequency, then at least the labeling should warn of this unexpected and undesired possibility. A rather simply designed study could answer this question and should include as controls, recipients of albumin prepared from venous sources individually tested for HBsAg by radioimmunoassay (RIA).

Albumin can tightly bind a wide variety of biological materials found circulating in blood or produced by placentas. Such physiochemical binding is one of its important biological properties. Placental starting material has more hemoglobin, blood group substances A and B, HL-A antigen and a wide variety of hormonal agents than venous plasma (Refs. 54 and 55). Women in labor in the United States are quite likely to have received a variety of drugs immediately before or during delivery. Some of these drugs would be expected to be bound to albumin in the placental source material. The fact that alkaline phosphatase of placental origin is found in albumin produced from placentas demonstrates that at least some of this material can be expected to remain attached to albumin or be carried through the fractionation process used in the United States and thus be present in the final product (Refs. 56 and 57). Small amounts of placental lactogen are also found in the final product, another demonstration of the same principle. Comparative studies suggest that the method used in the United States to remove excess hemoglobin from the placental source material is less effective than some alternatives in removing hemoglobin, blood group substances A and B, alkaline phosphatase, and transaminase (Ref. 58). The United States manufacturer reports undetectable levels of blood group substances A and B, and the level of hemoglobin meets current standards, but the marketed material is largely diluted with albumin derived from venous plasma, and placental alkaline phosphatase is still present in significant amounts. There are very few data available on the probability of various drugs being carried through the processing into the final product. Of these, penicillin might be the most likely one, but by no means the only one, with significant dangerous potential.

On the other hand, there is a long history of the use of placental albumin in the United States and other countries without recognition of significant clinical problems of the type discussed.

4. *Recommendations for Normal Serum Albumin*—a. Normal Serum Albumin (Human) should be placed in Category I.

b. The sodium content of all products should be limited to 130 to 160 mEq/L.

c. The Bureau of Biologics should be informed by the manufacturer of the amount of "reworking" on the production of each lot released.

d. Manufacturers using placental source material should demonstrate that penicillin is not carried over into the final product by performing a sensitive assay for penicillin allergen on a significant number of lots.

e. The manufacturer's production process should be designed to assure that the final product contains a maximal amount of albumin in the monomeric form. Current data suggest that this should be no less than 85 percent of the albumin in monomeric form at the time of release and no less than 75 percent of the albumin in monomeric form at the time of outdating.

f. *Labeling recommendations.* (1) The name of the product should be "Human Albumin." The terms "Normal" and "Salt Poor" should be discontinued in labeling. "Albumin" alone will suffice if albumin from other species is not

marketed in a form likely to be confused with this.

(2) Much current labeling and advertising do not reflect the best current information as discussed earlier. Albumin should not be promoted as a nutritional agent. When recommended to treat hypoalbuminemia, the labeling should also point out that improvement of the patient's nutritional status is a necessary and preferable approach, where possible. Administration of albumin in the shock phase within 24 hours after major burns is of questionable value as is its administration for cerebral edema. Specific diseases should be listed only as examples of broader, stated indications. Where specific circumstances apply, e.g., hemolytic disease of the newborn, this should be pointed out and appropriate references cited.

(3) More emphasis is needed in most labeling on:

(a) The potential for pulmonary edema with rapid administration of the 25 percent albumin product.

(b) Individualization of doses and rates of administration.

(c) Correcting the underlying cause of the condition requiring use of albumin.

(d) The hazards of use in patients with severe anemia.

(e) The potential for microbial contamination once the package is entered.

(4) Material prepared from placental sources should indicate in the labeling that a variety of biological materials may be present in small amounts which are not found in material from venous plasma.

(5) The approximate concentrations of chloride and potassium ions should be given in the package insert.

d. *Stability.* The current shelf-life expiration times may need to be revised as more studies are performed, and particularly if the requirement for minimum monomer content is adopted.

e. *Research*—(1) There is a great need for methods of measuring the dynamics and in vivo distribution of albumin; these methods must be more rapid and less cumbersome than those now available. This will allow needed studies to be carried out in acutely ill, unstable patients.

(2) It is still not clear at what levels and in what circumstances low concentrations of albumin in patients should be supplemented with albumin administered intravenously, and which are the best methods for doing so.

(3) The fate of albumin administered to acutely ill patients needs to be determined, especially for those patients with large losses of relatively sudden onset and those who are in markedly catabolic states.

(4) The precise relationship between concentration of albumin in the plasma and its effects on pulmonary function is still debated, especially when the lungs are damaged. This is an important clinical question that needs to be answered soon.

(5) The entire area of the clinical importance of the binding and carrier functions of albumin and the same functions of albumin products deserves considerably more investigative support and attention. Modification of regulations may be necessary as a result of such investigations if there are differences in clinically important binding and carrier functions that relate to methods of manufacture.

(6) The fate and function of albumin in monomeric, dimeric, and polymeric forms needs to be established so that proper criteria can be established for the manufactured product. The current Panel recommendation on monomeric limits are based on very incomplete data, but are regarded as reasonable interim standards based upon the results that current manufacturing practices can already achieve.

(7) The use of placental source material raises many potential problems because of the likelihood that a variety of substances are carried over into the final product. Albumin derived from placental source material needs to be tested for immunizing potential against HBsAg, placental lactogen, and perhaps other placental hormones. Drugs commonly administered to women in labor may be present in the final product, and their potential for harm needs to be evaluated. The immunizing potential of the small amounts of HL-A antigenic material in this product needs to be defined. Changes in regulations and labeling of placental albumin may be necessary, depending on the results of such studies.

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Product Reviews

There were 15 different suppliers of Normal Serum Albumin (Human) subject to this review, all with great similarity in the basic plasma processing procedures, but with many differences in details which conceivably could be significant. The products of each individual manufacturer are therefore described only with respect to obvious departures from common practices, along with comments on the labeling and advertising.

1. *Source.* Most manufacturers use Source Plasma (Human) and plasma from outdated whole blood. Both contain citrate anticoagulants and may have been frozen at least for transportation. Some manufacturers specifically state in their data submissions (Refs. 1 through 15) that each source unit was tested for hepatitis-B sulface antigen by radioimmunoassay and was free of such antigen. A few manufacturers are licensed to use placental material.

2. *Processing.* All manufacturers use the cold alcohol plasma fractionation process, described as the Cohn method 6, with many small variations. Placental material is handled by a somewhat different process.

3. *Composition.* The 5-percent (plus or minus 0.3 percent) solution of albumin is brought to volume with 0.15 M sodium chloride, with 0.004 M sodium acetyltryptophanate and 0.004 M sodium caprylate added as stabilizers. The 25-percent (plus or minus 1.5 percent) solution of albumin contains 0.02 M sodium acetyltryptophanate and 0.02 M sodium caprylate. None contain preservatives. All are at least 96-percent albumin, expressed as the percent of total protein present, with less than 3-percent globulins. Heme concentrations are less than 1 mg/5 grams albumin; pH is 6.9 plus or minus 0.5. All are sterilized by filtration, and heated to 60 °C plus or minus 0.5° for at least 10 hours. Final sodium content ranges from 100 to 160 mEq/L.

4. *Safety.* Lots and batches are visually checked during 7 days storage at 18 to 35 °C. Specified samples are randomly selected for culture for bacterial growth. All manufacturers check each lot in animals for febrile response and gross vasomoter reactions. Most manufacturers also check each lot in human volunteers for febrile response, effect on pulse rate and blood pressure, and symptomatic effects. All manufacturers keep records of reported reactions. Some manufacturers have voluntarily recalled lots associated with reported febrile responses. The commonest reported adverse reactions are chills and fever; much less commonly reported are tachycardia, hypotension which is rarely severe, urticaria, nausea, vomiting, increased salivation, and a skin rash. Transmitted diseases have not been documented to occur from properly processed material.

5. *Effectiveness.* No manufacturer has submitted effectiveness data for its specific product. All rely on the assumed similarity and homogeneity of this material in relation to that used in earlier studies. It is likely that some of the early published studies on effectiveness of albumin used material produced by methods similar to present methods in the Massachusetts Public Health Biological Laboratories.

6. *Labeling.* The bottle and package labels are all similar, including the product name, concentration of albumin, total volume, manufacturer's name and location, lot number, various other identifying numbers, United States License Number, osmotic equivalent as citrated plasma, date of expiration and added stabilizers. Instructions and warnings include: Temperature of storage, "contains no preservative", "do not use if turbid", caution against patient dehydration, "single dose container", "for intravenous use", the prescription legend and "see enclosed circular". All 25 percent albumin preparations were labeled "salt poor" at the time of the data submission, but recently published regulations require this designation to be removed.

The package inserts share several features in common. Indications for use include: Hypovolemia, hypoalbuminemia, and hemolytic disease of the newborn. Some inappropriate indications are mentioned below for individual manufacturers. Warnings, precautions and contraindications include cardiac failure due to hypervolemia, renewed bleeding as blood volume expands and perfusion improves, "dehydration" of the patient when 25 percent albumin is used as treatment for hypovolemia without supplemental fluids and electrolytes, severe anemia, and the fact that the material contains no preservatives and thus should not be used if turbid and should not be stored once opened. A statement is made that heat treatment at 60 °C for 10 hours probably eliminates the risk of hepatitis. Possible adverse reactions are described. Suggested doses for various clinical conditions are given. Essentially all of the information from the container and the package labels are usually repeated.

Advertising occasionally makes claims beyond those contained in the labeling. This is discussed where applicable in the review for each of the following products, realizing that manufacturers may have revised their labeling and advertising since the date of the submissions which were reviewed:

(1) *Abbott Laboratories (Albutein™)* (Ref. 1). A reasonable discussion in labeling of individual-specific dosing is present. The labeling also lists "adjunct in hemodialysis and cardiopulmonary bypass" as an indication for use. Advertising separates traumatic from hemorrhagic shock, mentions "ischemic (reversible)" and "stagnant (irreversible)" forms claims that early loss in "endotoxic" shock is plasma loss, and mentions cardiogenic shock as a distinct indication for administration of albumin. All of these statements are questionable if not objectionable. The indication for use of albumin in shock is hypovolemia; various theories of pathophysiology and categorization of shock are better avoided in labeling until more firmly established scientifically. This section of the labeling would be much better if so simplified and clarified. With respect to labeling for administration in burns, see the earlier Generic Statement on Normal Serum Albumin (Human). In discussing hypoproteinemia, correction of the underlying cause is correctly emphasized. Acute pancreatitis,

cerebral edema, and nutritional hypoproteinemia are mentioned as specific indications. It is concluded that the reviewed advertising for this product falls well below the quality of the package insert labeling and borders on being misleading.

(2) *Armour Pharmaceutical Company (Albuminar™)* (Ref. 2). The labeling does not list hemolytic disease of the newborn as an indication for use. It does list use in the shock phase which occurs immediately after major burns (see above). The labeling properly includes severe anemia as a contraindication.

(3) *Connaught Laboratories, Limited* (Ref. 3). The labeling specifies storage at 2 to 10 °C; therefore, it has a 5-year dating period. Apparently there is no testing in humans before lots are released. Connaught was no longer a licensed U.S. producer at the completion of this review.

Labeling is rather minimal. Hemolytic disease of the newborn is not mentioned as an indication for use, but burns are. Severe anemia is not specifically mentioned as a possible contraindication, and the lack of clotting components in this preparation is not pointed out. There has been no advertising in 5 years.

(4) *Cutter Laboratories, Incorporated* (Ref. 4). Burns and cerebral edema are mentioned as indications for use in labeling. Hemolytic disease of the newborn is not listed. Directions for use were not included in the package insert. There is separate packaging for material derived from blood collected from the American National Red Cross and paid for by the Red Cross. Advertising is minimal, but contains the potentially misleading statement that "cardiac output is greater than that produced by whole blood." Testing of lots in human volunteers is not described.

(5) *Dow Chemical (Proserum™)* (Ref. 5). Placental origin material was used until mid-1971. Menthanol is occasionally used as the precipitating alcohol. The process permits the product to be reheated up to five times to eliminate pyrogenic reactions.

The package insert for 25-percent albumin incorrectly suggests use in cerebral edema and use as a vehicle for delivering red cell concentrates as indications. Labeling for other indications has a more balanced presentation. The rise in alkaline phosphatase in recipients of the placental material is mentioned. The package insert for 5 percent albumin lists only hypovolemia as an indication.

Five and three lots of 25 percent and 5 percent albumin respectively have been tested in humans for adverse reactions. Two lots of 25 percent material were recalled in 1974 because of a reported incidence of adverse reactions in 17 patients. Dow Chemical was no longer a licensed producer at the completion of this review.

(6) *Hyland Division, Travenol Laboratories (Buminate™)* (Ref. 6). Production methods are very sketchily outlined in the submission to the Panel.

There are separate packages and labels for material produced for the New York Blood Center, and material for bulk use which is exported. The standard package insert points out lack of clotting factors in albumin and the inability to make absolute statements about the risk of transmission of viral diseases by the material. Hemolytic disease of the newborn is not mentioned as an indication for use. In general, the discussion is reasonably balanced. The precautions do not fully emphasize the necessary safeguards against bacterial contamination, and severe anemia is not mentioned as a contraindication. Instructions for use are detailed. Cerebral edema is incorrectly suggested as an indication for use with the 25-percent preparation. Hemolytic disease of the newborn is not mentioned.

(7) *Lederle Laboratories Division American Cyanamid (Pro-Bumin™)* (Ref. 7). Lederle has used placental starting material in the past, although reportedly this is no longer the case. The package inserts are adequate in their precautionary statements. Cirrhosis, nephrosis, and pancreatitis are listed as specific indications for use of the 25 percent preparation; the potential value of 25-percent albumin for cirrhotic ascites is overstated. In advertising, "prothrombin levels" are mentioned were "clotting factors" are meant; this may be confusing. The Panel disagrees with the statement that 25-percent albumin "has its greatest application in the treatment of shock." There has been some testing for safety in humans in the past.

(8) *Massachusetts Public Health Biologic Laboratories* (Ref. 8). Hemolytic disease of the newborn is not mentioned as an indication in the labeling. Starvation is mentioned twice; this should be updated to indicate currently preferred approaches to treatment of malnutrition. There has been no advertising.

(9) *Merck Sharp and Dohme, Division of Merck and Company, Inc. (Albumisol™)* (Ref. 9). Nutritive value is inappropriately mentioned in the package insert. Many specific diseases are mentioned under the usual broad categories of indications for use. Severe anemia is not mentioned as a contraindication. Detailed electrolyte content is given on some packages. Hemolytic disease of the newborn is not mentioned as an indication in most package inserts.

There were unusually large numbers of reactions reported in 1972 for the 5 percent product. Two of three lots were withdrawn and distribution of the third was discontinued. Retesting (not described) failed to reveal the cause.

(10) *Michigan Department of Public Health* (Ref. 10). Placental material has been used in the past. A questionable statement "at least 95 percent albumin" is used in the labeling. The package insert is very brief. Nutritional hypoproteinemia is inappropriately mentioned as an indication; hemolytic disease of the newborn is not mentioned. Precautions do not mention the possibility of inducing severe anemia. Each lot is reported to be tested in normal humans for adverse reactions. No adverse reactions were reported from clinical use.

(11) *North American Biologicals* (Ref. 11). The package insert inappropriately mentions effects on the pH buffer capacity of blood, which is minimal when compared with hemoglobin. Suggested use to provide a large mobile reserve of amino acids clearly has inappropriate nutritional implications. Hypoproteinemia of starvation and burns are also listed as indications

Various lots have been tested for adverse effects on over 100 patients and volunteers. No mention is made of adverse reactions in clinical recipients. No data were given on actual composition of the product. This firm was no longer a licensed producer at the completion of this review.

(12) *Ortho Diagnostics, Incorporated (Orthobumin™)* (Ref. 12). Acute pancreatitis and cerebral edema are inappropriately mentioned as indications for use. Four lots were reported to have been tested for safety in normal humans.

(13) *Osterreichisches Institut Fur Haemoderivate* (Ref. 13). All lots are reported to be tested in humans before release. There was no mention of reports of adverse reactions in patients. Cerebral edema and eclampsia which are included as indications in the labeling should be eliminated.

(14) *Parke, Davis and Company* (Ref. 14). Placental source material is used in combination with venous source material. Venous sources are tested for HBsAg, but individual placentas are not tested for HBsAg. Each lot is tested in patients before release. A rather large number of reactions were reported for 1970 without explanatory comment. Nutritional use as mentioned in advertising material should be

eliminated. Safety should be evaluated as recommended for placental source material in the earlier Generic Statement on Normal Serum Albumin (Human).

(15) *E.R. Squibb and Sons* (Ref. 15). The labeling precautions include possible elevation of alkaline phosphatase in recipients, even though the material is not of placental origin. The package insert is well balanced, although hints at nutritional advantages in anuric patients should be eliminated. No information was available regarding further testing, safety, and reported reactions in recipients.

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(3) BER Volume 6036.
(4) BER Volume 6016.
(5) BER Volume 6043, 6044 and 6045.
(6) BER Volume 6069 and 6069A.
(7) BER Volume 6019.
(8) BER Volume 6035A.
(9) BER Volume 6009.
(10) BER Volume 6042.
(11) BER Volume 6125.
(12) BER Volume 6085, 6086, and 6087.
(13) BER Volume 6101.
(14) BER Volume 6004 and 6004A.
(15) BER Volume 6031.

11. Plasma Protein Fraction (Human)

Generic Statement

Plasma Protein Fraction (PPF) was developed in 1957 (Ref. 1). The composition of the product is essentially that of Fraction IV-4 plus Fraction V which are obtained by method 6 developed by Cohn et al. (Ref. 2) to fractionate the proteins of human plasma. At the time of the development of plasma proteins during World War II only human serum albumin was available as a 25-percent concentration in 100 mL volume (Ref. 3). This solution often proved to be ineffective in restoring blood pressure without the addition of intravenous saline, especially in dehydrated patients. Thus, a 5-percent solution of plasma protein was readily received because of its ease of administration (Ref. 4). Also PPF preserves the national blood resources; there is 25 to 30 percent more yield of usable plasma proteinf solution from a unit of blood than when albumin is produced (Ref. 5).

Nearly all commercial fractionation methods are based upon the exploitation of solubility differences. Thus, the chemistry of protein ractionation is the chemistry of protein solubility. Protein solubility is affected by a number of variables, and if these variables are controlled, they can form the basis of systematic protein fractionation. In the fracttionatio of most natural protein systems, the presence of a large number of components and the similarity of many of them makes it necessary to control a number of variables if sufficiently large differences in solubility are to be obtained to give usefully precise separation.

One of the most difficult problems in protein fractionation is to avoid alterations of the proteins during their separation. Proteins are subject to irreversible alterations from many causes. The extent and nature of these alterations are often difficult to evaluate. In the fractionation of the proteins of human plasma, it is particularly important that unaltered proteins be made available for subsequent use in humans, since irreversible alteration might well lead to substances of new antigenic specificity if not more general changes in biological activity.

Production of PPF requires manufacturing methods that include low temperatures (−3 to −5° C) various concentrations of ethyl alcohol (8 to 30 percent), varying hydrogen ion levels (pH 4.6 to 7.2), and separation of precipitates. Virtually every step in the manufacturing process has the potential for causing protein denaturation, i.e., an alteration in the protein's molecular structure (Ref. 6).

PPF and hypotension. Other labile proteins may be altered by the fractionation process. For example, recent lots of PPF that produced hypotension were found to have high levels of prekallikrein activator (PKA), although most lots of PPF have low levels of this activator. PKA is perhaps better known as Hageman Factor Fragment (Table 1). Activated Hageman Factor can act to trigger blood coagulation and fibrinolysis, and also can activate prekallikrein to kallikrein which in turn acts upon high molecular weight kininogen to produce bradykinin (Table 2).

Table 1.—Some Components of the Kallikrein-Kinin System

1. Hageman Factor	Factor XII–MW about 80,000.
2. Prekallikrein Activator(s) or Hageman Factor Fragment(s).	"PKA"—MW about 28,000. Generated from Factor XII. Catalyze(s) Prekallikrein Kallikrein.
3. Plasma Prekallikrein.	"PK"—MW about 100,000.
4. Plasma Kallikrein	MW about 100,000. Catalyzes release of BK from HMWK. Has esterolytic activity
5. High Molecular Weight Kininogen(s).	"HMWK"—MW about 200,000. Preferred substrate for plasma kallikrein.
6. Bradykinin	"BK"—a nonapeptide, MW about 1,000. Has vasodilatory activity. Causes smooth muscle contraction

TABLE 2

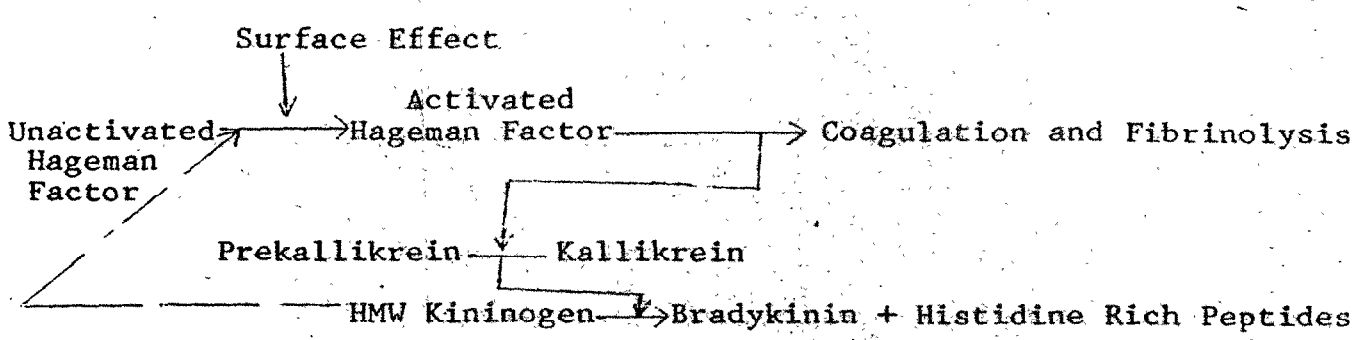

Diagram of the relationship of proteins of the kinin-generating pathway to the mechanism of Hageman factor.

Bradykinin is nonapeptide with a molecular weight of about 1,000 which has vasodilator activity and causes smooth muscle contraction. In the normal circulation, bradykinin is rapidly inactivated largely by kininases, proteolytic enzymes that degrade kinins to inactive forms. The half-life of bradykinin in blood is about 20 seconds; it is almost totally destroyed in one passage through the pulmonary circulation (Ref. 8).

It has previously been reported that during surgical procedures bradykinin levels increase from low (nanogram) levels to considerably higher levels (Ref. 9). If this occurs and another generator of bradykinin is added to the system (PPF) or if a kininase is removed from the system or the level of an inhibitor in this system is suppressed, there can be a resultant increase in bradykinin that can cause hypotension.

In the original work of Bland and his associates (Ref. 10) it was postulated that it was bradykinin that caused the hypotension produced by PPF. Recent studies at the Bureau of Biologics with samples of PPF retained from Bland's original studies were found to be high in PKA activity (Ref. 11). It has been shown that there is direct correlation between high levels of PKA and the amount of hypotension produced in animal models (Ref. 12).

In the "blocked rat" model, treated to inhibit kininases so that any bradykinin generated stays in the circulation, there is a linear correlation between the drop in mean arterial pressure and the concentration of PKA in the product administered. However, in unblocked rats it takes much more bradykinin, either generated or administered to produce a given fall in blood pressure, and the relationship tends to be much less linear (Ref. 13).

There is some evidence that there are higher PKA levels in salvaged plasma than in fresh frozen plasma, although there is not general agreement with this observation (Ref. 14).

PKA activity has been found in only a few lots of albumin. Indeed this is reasonably consistent because a further fractionation process is necessary to produce albumin, Fraction V; most of the PKA is removed by these additional steps. These protein fractions, however, are never so "clean" that the presence or absence of PKA can be absolutely predicted. PKA tends to concentrate in Fraction IV, but on rare occasions may be found in albumin which is made from Fraction V. After heating to 60 degrees for pasteurization bradykinin may still be present, kallikrein has been destroyed, kininogen is still present but at very low levels, and prekallikrein activator in present in various amounts and maintains its activities.

It is well known that the administration of PPF fractions do not cause adverse responses in all people. People have different prekallikrein, kininogen, and enzyme inhibitors in the circulation. Individuals vary in their ability to destroy bradykinin. They may also have differing levels of PKA because the trauma of surgery activates Hageman Factor.

There is evidence that ammonium sulfate as sometimes used in fractionation (Ref. 15) and cooling to a temperature of 4° C both activate Hageman Factor. These steps were used in the manufacturing process for PPF by one supplier until 1976. There is also the possibility that PKA could be generated in plasma which has been thawed to obtain cryoprecipitate (Ref. 16). Kallikrein can be rapidly inhibited by C 1 esterase inhibitor and by alpha 2-macroglobulin and more slowly by antithrombin III. Heating probably inactivates all these inhibitors.

Potentially, chemical reactions producing PKA can occur at almost any stage in plasma derivative manufacture, in the source plasma, during the fractionation, in partially fractionated products, in the paste, in the powder, in the bulk, and finally perhaps even on the shelf.

Composition of PPF. The protein composition of PPF is determined by methods that have been approved for each manufacturer. The protein content is set at 5.0 plus or minus 0.3 percent. In addition to the alpha and beta globulins present in the PPF (usually about 12 percent) the albumin exists at both a monomer and a dimer. The effect of albumin dimer on the oncotic characteristics and drug-binding properties of the albumin has not been determined. PPF is composed of at least 83 percent (usually about 88 percent) albumin, no more than 1 percent gamma globulin, 130–160 mEq/L of sodium, less than 2 mEq/L of potassium, and has a pH of 7.0 plus or minus 0.3. This solution is stabilized with 0.004 molor sodium acetyltryptophanate and 0.004 molar sodium caprylate. After being

introduced into the final container the PPF solution is heated at an attained temperature of 60°C plus or minus 0.5°C for 10 hours. The dating period is 5 years when stored at 5°C or 3 years at 30°C or less.

Evaluation of PPF for Labeled Indications

1. *Hypovolemia.* Hypovolemia, whether due to blood loss or "third space" sequestration of fluid (exclusive of burns) responds readily to PPF.

a. *Effectiveness.* PPF is effective in the maintenance of a normal circulating blood volume (Ref. 4), but has not been proved to be effective in the maintenance of oncotic pressure. The same labeling claims are made by all the manufacturers of PPF, although there may be possible differences in effectiveness among products relating to the methods of preparation. At the present time there is no information available about the effect of various methods of preparation of PPF upon the metabolism and oncotic effect of its albumin.

Precautions about PPF administration at a rate greater than 10 mL/minute may limit its usefulness as a rapid plasma volume expander when blood is not available unless efforts to reduce the hypotensive reactions are successful.

Much of PPF administration today is arbitrary or pragmatic. The advent of a clinical oncometer so that oncotic pressure could be measured before and after PPF administration should result in a better quantification of the effects of PPF and clarification of the need for its administration. These studies have not been done.

b. *Safety.* The safety of PPF has been assumed in the past to be so high that complications have already been reported even though some may have occurred. The reaction rate for various adverse side effects such as urticaria, chills, fever or vasodepressor effects are few and involve between 0.5 and 1.5 percent of the production lots. These reactions are for the most part mild and short-lived. The vasodepressor effects have been increasingly reported and may be significant in some patients.

As a result of the unusual stability of albumin, it is possible to heat the product routinely during processing to 60°C for 10 hours, thus achieving inactivation of hepatitis viruses. The only established cases of hepatitis after PPF infusion have apparently come from a failure in processing so that 60°C treatment for 10 hours was not achieved (Ref. 17).

c. *Benefit/risk evaluation.* Properly administered, PPF has an acceptable benefit-to-risk ratio. Until clarification of the frequency of occurrence and clinical significance of hypotensive episodes has been determined, an evaluation of the ultimate benefit-to-risk ratio for rapid, emergency administration cannot be achieved.

d. *Recommendations.* (1) Plasma Protein Fraction (Human) should be place in Category I.

(2) It is recommended that more sophisticated clinical studies be sponsored within the medical community to evaluate the effectiveness of PPF in elevating oncotic pressure possibly in conjunction with similar studies recommended for albumin. This information would clarify the many clinical disorders that exist in which there is incomplete or conflicting data on the effectiveness of PPF therapy and should define further the situations in which no benefit from PPF administration occurs, despite the presence of hypoalbuminemia.

(3) Manufacturers of PPF should have a control testing program for measuring PKA consistent with available technology.

(4) Acceptable levels of PKA should be no more than 25 percent of the present Bureau of Biologics reference standard.

(5) Limits for bradykinin content should be established when data are developed to substantiate measurable safe levels.

(6) The product name should be "Plasma Protein Fraction".

e. *Labeling recommendations for hypovolemia.* The package inserts should be revised to inform physicians of the possibilities of hypotension occurring after PPF administration and that this hypotensive effect can be reversed with the use of vasopressors. Also, the package insert should note that no evidence exists that PPF appreciably increases the colloid oncotic pressure of the plasma.

2. *Burns.* Acute therapy in burns is directed during the first 24-hours to the administration of large volumes of crystalloid solution to expand the constructed extracellular fluid volume. For continuation of therapy beyond 24-hours, albumin, often as PPF, and lesser amounts of crystalloids are generally used. Recommendations are the same as for hypovolemia.

3. *Red cell resuspension medium*—a. *Effectiveness.* During certain types of exchange transfusion or when very large volumes of packed red blood cells have been administered to correct blood loss, PPF may be used as a resuspension menstruum in order to avoid excessive hypoalbuminemia during such transfusion therapy. Although such blood is deficient in coagulation factors and platelets, sufficient equilibrium exists between intravascular and extravascular clotting factors to maintain adequate hemostasis during blood exchanges of less than 90 percent.

b. *Safety.* If circulatory overload is avoided and if it is realized that PPF has no plasma-clotting factors, then adequate levels of albumin can be achieved by the resuspension of packed red blood cells, washed red blood cells or previously frozen red blood cells during massive transfusion procedures.

c. *Recommendations.* Recommendations are the same as for hypovolemia.

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12. Antihemophilic Factor (Human)

Generic Statement

1. *Introduction.* Antihemophilic Factor (AHF, factor VIII, antihemophilic globulin, AHG) is generally considered to be a trace protein in normal plasma which has the property of correcting the clotting defect characteristic of hemophilia A (Ref. 1). Recent work indicates that hemophilia A is not a single disease entity in which there is a deficiency of a protein which has coagulant activity. Factor VIII-like antigenic activity can be identified independent of factor VIII coagulant activity (Ref. 2). In addition, von Willebrand's disease is characterized by several plasma defects in addition to a deficiency of factor VIII coagulant activity (Ref. 3). Studies on genetic control mechanisms, synthesis and physical-chemical composition of factor VIII have a relationship to the effectiveness of therapy with factor VIII concentrates in specific disease entities (Ref. 4 through 6). However, potency of the licensed Antihemophilic Factor (Human) products is characterized on the basis of factor VIII coagulant activity. Unless otherwise specified in this review, the term factor VIII relates to only the clot-promoting properties of this protein.

Early attempts to isolate the antihemophilic activity from plasma were limited by the techniques for its assay and failure to recognize that hemophilia was not a single disease entity. It was found that plasma globulin fractions had the ability to correct the prolonged whole blood clotting time characteristic of severe hemophilia (Refs. 6 and 7). The antihemophilic activity was found to separate along with fibrinogen in fraction I of the ethanol precipitation method of Cohn, et al. (Ref. 8). The factor VIII activity of fraction I preparations was variable but it was used in the clinical management of hemophilia (Ref. 9). At the present time, no fraction I preparations are licensed for use in the treatment of hemophilia. A number of other procedures have been used for separation of factor VIII activity from plasma of humans as well as from the plasma of other species (Ref. 1). Work has been done in England on factor VIII from porcine and bovine plasmas (Ref. 10). Although high potency preparations have been obtained, the porcine and bovine materials have been found to be antigenic when used in man (Ref. 11).

Pool and associates (Ref. 12) recognized the potential of what was first regarded as a laboratory curiosity and their observations provided the basis for the materials that are currently licensed. When plasma is frozen and thawed, much of the factor VIII activity is concentrated in a small amount of poorly soluble material. This cryoprecipitate can be separated from the supernatant plasma and is the starting material for currently licensed factor VIII concentrates (Ref. 13). As with other fractionation procedures, there is also a considerable amount of fibrinogen in the cryoprecipitate.

Factor VIII is relatively stable when separated from other procoagulants but small amounts of thrombin will destroy it (Ref. 1). In order to remove procoagulants which might provide a source of thrombin, aluminum hydroxide has been found to be effective (Ref. 1). Aluminum hydroxide absorbs fibrinogen from plasma to a greater extent than factor VIII (Ref. 4). Thus much of the fibrinogen present in cryoprecipitate can be removed by this method. Since the aluminum hydroxide is insoluble it can be removed by centrifugation. Aluminum hydroxide, like other absorbants, will remove a number of other clotting accelerators that influence in vitro test results but have no in vivo antihemophilic activity. It is probably for this reason that there is good correlation between in vitro test results of factor VIII. potency and the clinical effectiveness of the products prepared by this procedure.

Studies done on patients undergoing hemodialysis have indicated that aluminum is potentially toxic (Refs. 15 and 16). If aluminum hydroxide is used in the process of manufacturing factor VIII, the product must be tested to demonstrate that the aluminum has been removed.

In some procedures for the isolation of factor VIII small amounts of heparin are added (Ref. 17). This is apparently used to prevent the activation of clotting factors which would lead to thrombin formation. When heparin is added during the manufacturing process, the product must be tested to demonstrate that heparin has been effectively removed, or the residual heparin activity should be measured and indicated in the labeling.

Intermediate purity AHF (Ref. 18) is a term applied to a product in which the cryoprecipitate obtained from plasma is treated with ethanol. The cryoethanol precipitate is extracted with low ionic Tris buffer and then treated with aluminum hydroxide.

Glycine will precipitate factor VIII. Low concentrations of glycine, 3.5 percent, will precipitate nonfactor VIII proteins, mainly fibrinogen, while most of the factor VIII activity remains in the supernatant fluid. When the concentration of glycine is increased to approximately 12 percent, most of the factor VIII will precipitate. The factor VIII activity can then be extracted with a citrate solution (Ref. 19). In a similar manner polyethylene glycol (PEG) can be used as a precipitating agent (Ref. 18). At low concentrations of PEG, 2.5—5.0 percent, nonfactor VIII proteins will precipitate, while at the higher concentration of 2 percent, factor VIII will precipitate. The factor VIII can be recovered by extraction with Tris buffer. These preparations have been termed high purity AHF (Ref. 18).

2. *Indications for use.* Factor VIII concentrates are indicated when there is a hemostatic defect resulting from a deficiency of factor VIII, and it is necessary to increase and maintain factor VIII concentrations to control bleeding.

a. *Evidence of effectiveness.* (1) There is a considerable amount of available information to indicate that factor VIII when given in adequate amounts will correct both the clotting and hemostatic defects associated with factor VIII deficiency. The active material has a relatively short effect in vivo (Ref. 20) and approximately half of the coagulant activity is lost from the circulating plasma in 10 to 12 hours. In order to

maintain circulating factor VIII at therapeutic levels, additional material must be injected at approximately 12-hour intervals (Ref. 12). The currently available materials are effective only when administered intravenously

(2) In disseminated intravascular coagulation (DIC) there is no evidence that factor VIII concentrates are therapeutically useful.

(3) A small percentage of patients with hemophilia A develop antifactor VIII anticoagulants (Ref. 22). This type of anti-factor VIII activity appears to be an antibody response to factor VIII-like antigenic material that has been administered. When this complication occurs, massive amounts of factor VIII must be supplied and even this may not be sufficient to achieve hemostatic effectiveness (Ref. 19). It is important that the in vivo effectiveness of the factor VIII material be determined by a reliable assay procedure since it is not always possible to predict in vivo effectiveness from the vitro test results.

(4) Acquired factor VIII deficiency has been reported in otherwise normal individuals following reactions to penicillin, during pregnancy, in patients with lupus erythematosus and in patients with rheumatic heart disease (Ref. 22). In such cases an anti-factor VIII anti-coagulant is apparently the basis for the coagulation defect. There may be little or no in vivo response to administered factor VIII in these patients. It is necessary to have a reliable method to evaluate the in vivo effectiveness of the infused material before Antihemophilic Factor (Human) can be evaluated for this indication.

(5) Von Willebrand's disease is a disorder in which the affected individuals have decreased levels of factor VIII, a prolonged bleeding time and other recently recognized plasma activity deficiencies (Ref. 3). The relationships among the plasma abnormalities, the hemorrhagic tendency and the genetic defect are not clear. The adminstration of normal plasma or cryoprecipitated antihemophilic factor appears to control hemorrhage, normalize the bleeding time, and increaase the circulating level of factor VIII. The response of these patients to adminstration of normal plasma is unusual in that a secondary increase in circulating factor VIII occurs over and beyond the amount of factor VIII infused. In two recent studies (Refs. 4 and 5) the response of patients with von Willebrand's disease to administration of cryoprecipitated antihemophilic factor and two of the Antihemophilic Factor (Human) concentrate products was determined. Cryhoprecipitated antihemophilic factor increased the factor VIII coagulant activity, the factor VIII antigen-like activity, the platelet aggregating factor, the ristocetin related activity of platelets, corrected the bleeding time and controlled bleeding. In equilvalent doses of factor VIII, based on coagulant activity, the concentrates failed to correct the bleeding time, and clinical evidence of hemorrhage persisted even though high levels of factor VIII coagulant activity were achieved. In view of these findings, until additional data to the contrary are available, none of the Antihemophilic Factor (Human) concentrates can be considered to be effective in controlling bleeding in von Willebrand's disease.

(6) Most commonly Antihemophilic Factor (Human) is used when a patient with a known factor VIII deficiency has an episode of bleeding or when a surgical procedure is to be done. In some patients it has been found to be advantageous to administer factor VIII prophylactically. The rationale for such therapy is that certain individuals are particularly prone to develop bleeding episodes which can be minimized by maintaining factor VIII at relatively low levels. Such therapy is indicated in a few individuals that are carefully selected on the basis of clinical history.

b. *Evidence of safety.* (1) Since pooled human plasma is the source material for Anti-hemophilic Factor (Human), the risk of hepatitis is present. Current regulations required that each donor must be tested and found nonreactive for hepatitis B surface antigen (HBsAg) by "third generation" methods. This is a relatively new requirement, and its effect on the incidence, of associated hepatitis associated with the use of factor VIII is not yet clear. However, at the present time it must be considered that these materials carry a definite hepatitis risk.

(2) Anti-A and anti-B group antibodies are present in Anti-hemophilic Factor (Human) prepared from plasma containing these antibodies. These antibodies do not appear to be concentrated along with factor VIII. When large amounts of factor VIII concentrate are given, these antibodies can cause a mild hemolytic response in the recipient whose red blood cells have the appropriate antigens (Ref. 23). The reactions reported to date have not been serious, but if this possibility is not recognized, the resulting decrease in red cell volume may be misinterpreted as evidence of bleeding.

(3) Mild allergic-type reactions have been observed following the adminstration of Antihemophilic Factor (Human), but these reactions are uncommon.

c. *Benefit-risk evaluation.* When used appropriately to increase the circulating factor VIII coagulant level, the risk is small compared to the benefits to be expected.

3. *Recommendations.* a. The licensed Antihemophilic Factor (Human) products should be placed in Category I since when used appropriately they are relatively safe and effective.

b. Studies should be encouraged within the medical community to provide a source of AHF which is free of the hepatitis agent(s).

c. Further research is needed to determine how to provide information about the content of anti-A and anti-B antibodies in the final product. Although these antibodies are present in trace amounts, they may be clinically significant when massive doses of the product are given to patients. On the basis of current information, the labeling should indicate this possibility. The manufacturers should test each final lot of the product to determine the amount of anti-A and anti-B antibodies in order to detect unexpectedly high levels in relation to those established in the product license. Upper limits should be established if adverse reactions are discovered to relate to specific antibody levels.

d. If aluminum hydroxide is used in the process, the final product must be demonstrated to contain no more than 0.1 microgram of aluminum per unit of antihemophilic factor.

e. If heparin is used in the process, the final product must be demonstrated to be free of heparin activity, or the amount of heparin activity remaining should appear in the labeling.

f. The labeling should contain information about the fibrinogen content of the final product, cautions about the risks of hepatitis and anti-A and anti-B antibody content, and a statement that the product is not indicated for the treatment of von Willebrand's disease.

g. The product name should be "Antihemophilic Factor".

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13. Factor IX Complex (Human)

Generic Statement

1. *Introduction.* Factor IX deficiency (Hemophilia B) is a sex-linked hereditary coagulation disorder. It is approximately ¼ as common as factor VIII deficiency (Hemophilia A) (Ref. 25). Like factor VIII deficiency, it is a heterogenous disorder with several variants. In some patients factor IX antigen is detected by showing an immunological reaction between the patients' plasma and either a spontaneously occurring factor IX antibody or one prepared in rabbits by injecting partially purified factor IX concentrates; this is called Hemphilia B+. Those disorders in which a true deficiency exists, i.e., a factor IX protein [active or inactive] cannot be detected, are called Hemophilia B−. In a total of 179 patients studied by six different investigators, the incidence of Hemophilia B+ has been reported as between 8 and 46 percent. Much of this wide range of incidence may be due to differences in techniques. In addition, there are a small numberof patients with a variant called Hemophilia Bm. These individuals have an abnormal prothrombin time (PT) when tested with ox brain thromboplastin, suggesting that an abnormal protein is formed in these patients which interferes with the PT determination. Every combination of Hemophilia B+ or B− with Bm+ or Bm− has been reported.

Patients with factor IX deficiency can be treated with plasma infusions to control or prevent their bleeding problems. Because of difficulty in achieving levels of factor IX greater than 20 percent by plasma infusions without overloading the circulation, techniques for concentrating factor IX have been developed. Most of the techniques have taken advantage of the absorbant qualities of factor IX, as well as the other prothrombin complex factors (II, VII, X), to materials such as: tricalcium phosphate (Ref. 3), barium sulphate (Ref. 4) DEAE cellulose (Ref. 5), DEAE sephadex (Ref. 6) or aluminum hydroxide (Ref. 7). Recent modifications of the procedures have allowed preparation of factor IX concentrates from residual citrated plasma obtained as a byproduct of routine blood banking.

There are currently two United States licensed producers of factor IX concentrates. These preparations also contain factors II, VII and X. Some of the currently used European products do not contain factor VII. Of the American Factor IX Complex (Human) products, one is marketed under the trade name of Proplex™ (Hyland Laboratories) and the other as Konyne™ (Cutter Laboratories).

2. *Effectiveness*—a. *Congenital deficiencies of factors II, VII, IX or X*—(1) *Prophylaxis and treatment of bleeding disorders.* A general review of the clinical uses of factor IX concentrates by Menache (Refs. 8 and 9) provides data on products similarly prepared to those commercially available in the United States. These studies showed that factor IX concentrates were successfully used to treat hemorrhagic episodes or to prevent bleeding with major surgery in patients with deficiencies of factors II, VII, IX and X.

b. *Patients with inhibitors*—(1) *Factor IX inhibitors.* The reported incidence of these inhibitors varies widely, from 9 to 50 percent (Ref. 10). They have been found to be IgG antibodies and usually do not significantly increase the bleeding tendency of the patient. When tested in vitro they are found to be much more rapidly acting than factor VIII inhibitors. If patients with factor IX inhibitors are treated with factor IX-containing material, the antibody is eventally recalled in high titer.

(2) *Factor VIII inhibitors.* Breen and Tullis (Ref. 11) noticed shortening of the clotting time out of proportion to the elevation of clotting factors in patients with vitamin K deficiency who were infused with a clotting factor concentrate prepared by absorption on DEAE cellulose. In order to evaluate this post-transfusion hypercoagulability, a patient with factor VIII deficiency with bleeding was infused with the factor IX concentrate. Prompt cessation of the bleeding occurred with no change in the factor VIII levels. Subsequent reports (Refs. 12 through 14) have indicated that some factor IX concentrates may be able to bypass the inhibitor and provide

hemostasis. Tishkoff (Ref. 15) has, confirmed the presence of variable quantities of IXa, Xa and thrombin in these products and demonstrated that they all shorten the activated partial thromboplastin time (PTT) of normal plasma. Whether this provides an explanation for their beneficial effect remains to be determined.

A recent report by a task force on the clinical use of factor IX concentrates (Ref. 16) generally agreed that the IX concentrates available at that time may have been useful in the treatment of factor VIII inhibitors but indicated that a multi-institutional study was needed to establish their exact role in treatment of this problem.

c. *Multiple coagulation factor deficiencies*—(1) *Coumarin overdosage.* Factor IX concentrates have been used successfully in the treatment of coumarin overdosage of for reversal of anticoagulant therapy in patients prior to surgery (Ref. 17).

(2) *Liver disease.* Factor IX concentrates have been given to large numbers of patients to treat the hemorrhagic diathesis associated with liver disease. The results have not always been successful. In some patients this may be because of other factor deficiencies not corrected with factor IX concentrates such as factor V (Ref. 17) or because of the high incidence of bleeding due to local lesions such as varices or ulcers.

In addition, two other major concerns have arisen about treating patients with liver disease with factor IX concentrates. These are the high risk of post-transfusion hepatitis found after giving factor IX concentrates superimposed on an already damaged liver and a hypercoagulable syndrome with thromboembolism first reported in 1959 (Ref. 18). Because of this latter observation one of the two factor IX products available in the United States also contains added heparin. Some authors originally reported no evidence of an increased incidence of thromboembolism after administration of these materials to patients with chronic liver disease (Ref. 19 through 22). However, other studies have shown a risk of thrombosis or induction of disseminated intravascular coagulation (DIC) in patients with liver disease when these materials were used (Refs. 8, 23, 24, 26 through 28 and 43). Blatt described a patient with congenital factor X deficiency who developed hepatitis following adminstration of Konyne™ (Ref. 23). A subsequent surgical procedure was required while the patient still had abnormal latex fixation tests (LFT's), and within 2 hours of her second dose of this product she died with diffuse thromboembolism. Cederbaum reported three patients with liver disease who developed DIC after infusions of Proplex™ (two patients) and Konyne™ (one patient) (Ref. 24). Hoag described two patients who developed evidence of fibrinolysis following administration of Konyne™ (Ref. 6). Kasper reported an additional four patients with chronic liver disease who developed DIC following factor IX concentrates (Ref. 26). Similar reports have come from Europe after the use of PPSB (Ref. 8). A factor IX concentrate prepared in Great Britain was associated with acute liver disease (Ref. 27). The high risk of DIC and thrombosis in these patients may be related either to failure of the role that the liver normally plays in clearing activated products of blood coagulation (Ref. 28) or, alternatively, some of these patients may already have had underlying DIC which is exacerbated by this therapy.

(3) *Newborns.* Low birth weight infants have been shown to have a high incidence of intracranial hemorrhage. If the thromboplastin generation test on the first day of life is less than 10 percent, the incidence of hemorrhage is three times greater than in those infants with activities greater than 10 percent (Ref. 29). However, in a controlled trial where factor IX concentrates were administered intraventricular hemorrhage was three times greater in the treated group (Ref. 30). One study has indicated a reduction in intercerebral hemorrhage in infants with respiratory distress syndome (RDS) who required assisted ventilation from 33 percent to 13 percent when the infants were treated with factor IX concentrates (Ref. 31). Other studies have shown that in newborns with infection, RDS, hyaline membrane disease or vitamin K deficiency, use of factor IX concentrates has produced DIC (Ref. 8).

(4) *Open heart surgery.* There are no studies which have evaluated the efficacy of factor IX concentrates in the control of bleeding in open heart surgery patients. However, these concentrates are being used in this situation as documented by a report of a very high incidence of hepatitis following open heart surgery in a group of patients who received Konyne™ (Ref. 32).

3. *Side effects*—a. *Hepatitis.* A high incidence of hepatitis has been reported following the use of factor IX concentrates with reports ranging from 28 to 70 percent (Ref. 33). Most of the patients tested were hepatitis B positive. In a report by Sandler in 1973, a lot of Konyne™ implicated in three cases of hepatitis could not be shown to be HBsAg positive by immunodifussion, immunoelectrophoresis and immunoelectrosmophoresis (Ref. 34). Iwanson's study (Ref. 33) reported two eras of this treatment: (1) 1969–1970 when patients were treated with Konyne™ or a Swedish concentrate and the hepatitis incidence was 9/26 or 35 percent, and (2) since 1971 when an additional 26 patients were treated with the Swedish concentrate with a hepatitis incidence of 2/26, or 8 percent. The reported difference may have been related to hepatitis testing by radioimmunoassay (RIA) of the source plasma since 1971. However, a more recent study reported in 1977 examined samples of Konyne™ and Proplex™ and showed that Proplex™ was positive for HBsAG by radioimmunoassay (RIA) and immune-electron microscopy (IEM), and Konyne™ was found to be positive by IEM. These data suggest that even though routine screening of each unit of plasma by RIA is performed before fractionation, this procedure may not be sufficient to protect recipients against hepatitis transmission by these products (Ref. 35).

b. *Thromboembolic events.* Thromboembolic complications of factor IX concentrates have already been discussed. In addition, there is considerable evidence to show that patients who have congenital deficiencies of factor IX without liver disease when treated with factor IX concentrates have an increased incidence of post-operative thromboembolic phenomenon. There are also scattered reports of thromboembolic problems following administration of factor IX concentrate without apparent precipitating events.

In a review article on this subject, Kasper (Ref. 26) reported only two factor IX deficient patients without liver disease or surgery who developed any such problems. One had a syncopal episode while being treated for a factor IX inhibitor, and the other had pulmonary emboli on three different occasions while being treated for muscle-skeletal bleeding. In a recent conference on thrombogenicity of factor IX concentrates the following data were provided, as shown in Table 1 (Ref. 36):

TABLE 1.—THROMBOGENICITY OF FACTOR IX CONCENTRATES

	Post-surgery	Nonsurgical	Total
Christmas disease	[1]4/21	2/85	6/106
Christmas disease and liver disease	0/6	4/18	4/24
Liver disease	1/3	9/55	10/58
Total	5/30	15/158	20/188 (11%)

[1]Number of patients with thrombosis/number treated.

The factor IX concentrates prepared in Europe have been reported to cause fewer complications. However, Preston (Ref. 37) infused the concentrate prepared in England into stable factor IX deficient patients and showed significant decreases in factor VIII and serum antithrombin III in all five patients studies; fibrinogen fell in four, and platelet counts decreased in two. These changes were transient, and there were no detectable clinical sequela. The study does suggest that more careful monitoring of hemostatic parameters in patients receiving these materials might show further evidence of thrombogenicity. There is one report of DIC occurring in a post-operative factor IX deficient hemophiliac which was successsfully treated with heparin; subsequently, DIC did not recur when the lot of concentrate was changed (Ref. 38) Sas (Ref. 39) described studies of the concentrate prepared in Scotland and showed that all lots of diluted concentrates on recalcification generated thrombin. Similarly, studies by Kingdon (Ref. 40) showed that materials prepared by eleven different manufacturers varied considerably regarding their thrombogenic potential as measured in vitro by a PTT assay and in vivo in a rabbit venous stasis model. The in vitro assay was more sensitive, but the in vivo assay may correlate more closely with the in vivo experience in man. Addition of 5 to 10 units of heparin/mL of reconstituted concentrate eliminated thrombus formation in the experimental animal model. However, a soybean trypsin inhibitor which inhibits factor Xa and diisopropyl fluorophosphate, an inhibitor of thrombin, VII or XIa has no effect.

Another approach to prevention of thrombogenicity by factor IX concentrates has been to add heparin in the manufacturing process to prevent factor activation and then provide for heparin removal in latter manufacturing steps (Ref. 41). This material was shown not to shorten in vitro clotting times.

4. *Benefit/risk evaluation.* Because of the high risk of hepatitis and thromboembolic complications, the use of these materials should be restricted to situations where the patient cannot be appropriately managed with single donor plasma. These would include: (1) Major surgery in patients with severe congenital deficiencies of the clotting factors contained in these concentrates, (2) life-threatening bleeding in patients with factor IX inhibitors, and (3) prophylaxis for patients with congenital clotting factor deficiencies where the value of this treatment must be weighed against the risk of hepatitis which is especially great in patients who have had few prior transfusions (Ref. 42). Factor IX concentrates should ordinarily not be used to treat bleeding during open heart surgery or for prophylactic treatment of patients with congenital deficiencies of prothrombin complex factors who also have liver disease.

5. *Recommendations.* Generically, Factor IX Complex (Human) should be placed in Category I. Specific recommendations for each producer follow in separate product reviews. Other recommendations for further research are:

a. Changes in manufacturing which can eliminate production of activated coagulation factors and contamination with hepatitis virus should be investigated.

b. Quality control technique improvements and better in vitro tests are needed for use by the manufacturers to lead to a material that is as nonthrombogenic and free of hepatitis as is possible.

c. Studies are needed to investigate the incidence of hepatitis in recipients who have received concentrates prepared from plasma pools composed of individual units which were HBsAg negative by third generation testing.

d. Studies should be encouraged to provide a source of factor IX which is free of hepatitis agents.

e. Further evaluation of the usefulness of factor IX concentrates in the treatment of patients with factor VIII inhibitors is recommended.

6. *Labeling recommendations.* a. The activity in the final product of any of the prothrombin complex factors (II, VII, IX, X) for which administration of the product is recommended as therapy must be included in the labeling.

b. The quantity of sodium, potassium, and anticoagulants in the final product must be included in the labeling.

c. The labeling should include prominent warnings about risks of hepatitis and thrombosis.

d. Indications for use should be restricted as discussed in this report.

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Product Reviews

1. *Product.* Factor IX Complex (Human) manufactured by Cutter Laboratories, Inc., as "Konyne™" is supplied as a lypholized powder containing 500 units of factor IX per bottle. An isotonic solution for administration is made by reconstituting the powder with 20 mL sterile water (USP) supplied by the manufacturer. The product is given by intravenous administration through a filter. No preservatives are used. Other ingredients are Factors II, VII, and X in amounts roughly proportionate to their respective levels in fresh plasma. The product should be stored at 2 to 8 1°C. For travel purposes, it may be stored for up to 1 month at temperatures not to exceed 37 °C.

In an analysis of the product, Tullis (Ref. 1) reports that Konyne™ contains:

TABLE I.—KONYNE™ Components

Substance	Amount	Concentration times plasma protein
Factor IX units [1]	460–550	70X
Factor IX Specific Activity (units/mg protein).	1.1	
Factor II units [2]	140	7X
Factor II Specific Activity	0.28	
Factor VII		36X
Factor X		30X

[1] One unit=factor IX activity of 1 mL of pooled plasma.
[2] One unit=prothrombin activity of 1 mL fresh plasma (not NIH prothrombin units).

2. *Effectiveness*—a. *Treatment of deficiencies of factors II, VII, IX and X (congenital and acquired).* In studies of factor IX deficiency, six patients received 62 transfusions with Konyne™ with a post-transfusion recovery of 58 percent of the factor IX infused. Half-life of the first component was 4.6 hours and of the second 31.5 hours. Equilibration between the first and second component was 22.1 hours, and the volume of distribution was 2.7 times the plasma volume (Ref. 2). These distribution and turnover rates were the same as those found for fresh frozen plasma in three of the patients. Increases of factor II in these patients ranged from 62 to 299 percent, factor VII from 53 to 180 percent, and factor X from 72 to 232 percent. Factor II returned to control levels in 2 to 4 days, factor VII in 12 to 30 hours, and factor X at 5 days.

Tullis describes treating 14 patients with Konyne™ on 89 occasions and with Proplex™ on four occasions with clinical benefit in all instances. Diagnoses of the treated patients were: factor IX deficiency (6 patients), liver disease (6 patients), Dicumerol induced hypoprothrombinemia (2 patients), and factor VIII deficiency (1 patient) (Ref. 1).

Cutter Laboratories reported results of studies of the deficiency states and treatments shown in Table II (Ref. 20).

TABLE II.—REASONS FOR TREATMENT OF PATIENTS WITH DEFICIENCIES OF FACTORS II, VII, IX AND X

Factor deficiency	Surgery, post-surgery	Bleeding episodes	Hemarthrosis	Dental work	Prophylaxis	Assay	Total number treatments
II	0	0	0	1	0	0	1
VII	2	3	0	1	2	5	13
IX	22	59	13	6	5	21	126
X	1	3	2	0	0	0	6
Totals	25	65	15	8	7	26	146

In vivo recovery of factors II, VII, and X in factor IX deficient patients reported by Cutter Laboratories are shown in Table III (Ref. 20).

TABLE III.—IN VIVO RECOVERY OF FACTORS II, VII, AND X IN FACTOR IX DEFICIENT PATIENTS

Deficient Factor	II	VII	X
Number of cases	24	21	23
Increase as %/unit of factor IX infused/lb	15.5	5.1	7.3

For factor IX deficient patients, administration of 2 units of factor IX activity/kg body weight resulted in an immediate increase of 3 percent (range 1.7 to 5.0 percent) in factor IX levels. The same dosage resulted in an increase in factor VII of 4 percent (range 2.5 to 5.4 percent).

In the one factor II deficient patient studied, 73 units of factor IX infused/kg resulted in an increase from 1.5 percent to 185 percent of normal factor II.

In vivo results of therapy in factor X deficiency were found to be somewhat variable. To raise the circulating level by 1 percent, 0.35 to 3.9 units/kg were required (3 studies).

Other studies summarized in Table IV showed increases in in vivo response in severely deficient patients (Ref. 20).

TABLE IV.—IN VIVO RESPONSE IN SEVERELY DEFICIENT PATIENTS

[Congenital and acquired deficiencies]

Deficient factor	Condition	No. cases	Average % increase/unit of factor IX infused/lb.	Range
IX	Stable	17	3.2	1.7–5.1
IX	Bleeding	24	4.6	1.7–9.6
VII	Stable	3	5.2	5.0–5.4
VII	Bleeding	8	5.7	2.5–8.4
II	do	6	5.0	2.7–7.4
X	do	7	4.7	2.1–8.6

b. *Treatment of patients with inhibitors*—(1) *Factor IX.* One report has described successful dental extractions in patients from one family with inhibitors of factor IX by using Konyne™ to neutralize the inhibitor and levate the factor IX level (Ref. 3).

(2) *Factor VIII.* Abildgaard (Ref. 4) has reported the use of Konyne™ in 5 hemophilia A patients with inhibitors to treat 84 bleeding episodes with 130 infusions. Prompt control of bleeding was observed in each instance with doses of 15 to 10 units of factor IX/kg. No complications were encountered.

c. *Multiple factor deficiency*—(1) *Coumarin overdosage.* Konyne™ has been used successfully to reverse bleeding secondary to coumarin overdosage in 13 patients; in 5 patients reversal of coumarine effect was required before surgery; and 1 patient was treated for vitamin K deficiency before a biopsy procedure (Refs. 1, 5, 6, and 7).

(2) *Liver disease.* Liver disease has been discussed earlier in the Generic Statement on Factor IX Complex (Human).

3. *Safety analysis.* a. In 163 courses of treatment in 113 patients, Cutter (Ref. 20) reports that 22 patients had a temperature rise above 99.6° F, 4 patients had chills, 3 patients had allergic reactions (hives and itchy eyes), 1 patient had nausea, 3 patients with liver disease had fibrinolytic reactions, and there were 15 deaths (1 patient with thrombosis, 13 in patients with multiple coagulation factor deficiencies and 1 patient with sepsis).

b. *Hepatitis.* Kasper reported that 3 of 10 B hemophiliacs over 4 years old who had received fewer than 10 prior transfusions of blood products developed hepatitis within 6 months of the first infusion of Konyne™ (Ref. 10). Other hepatitis reaction reports in patients treated with Konyne™ are summarized in Table V.

TABLE V.—HEPATITIS IN KONYNE™ Treated Patients

Author	No. hepatitis/No. treated	Disease
Hellerstein (6)	1/2	Factor IX deficiency.
	2/4	Warfarin overdosage.
Faria (11)	6/9	Cardiac surgery (No Konyne™ with cardiac surgery hepatitis incidence was 1/23).
Oken (12)	2/4	Factor IX deficiency.
	1/2	Coumarin overdosage.
	2/2	Cirrhosis.
Sandler (7)	1/8	Coumarin overdosage.
	3/4	Coumarin overdosage with other blood products.
Grammens (5)	3/15	Factor IX deficiency.
	1/12	Coumarin overdosage or hepatic failure.
Boklan (13)	4/10	Factor IX deficiency.
Iwanson (14)	2/26	Do.

Ten patients were studied by bilirubin, SGOT, and SGPT liver function tests following Konyne™ administration, Table VI (Ref. 20). In three, there were abnormalities noted in the liver function tests, two associated with clinical hepatitis and one with transient enzyme changes without overt disease which was considered to be subclinical hepatitis. One additional patient had clinical hepatitis at the time of starting therapy, and this subsided during treatment.

c. *Thrombosis.* Triantaphyllopoulos (Ref. 15) initially described intravascula generation of thrombin in rabbits given Konyne.™ Death occurred in 34 percent of the treated animals. Catheter injection of the material was lethal in 82 percent of the animals, while direct intravenous injection was associated with a 13-percent mortality. These differences may be related to further contact activation of the Konyne™ by the catheter during infusion. Kasper has reported that 6 of the 13 factor IX deficient patients in the post-operative period who were treated with Konyne™ developed thromboembolic problems (5 thrombophlebitis and 1 pulmonary embolism) (Ref. 16). Steinbert (Ref. 17), Edson (Ref. 18), and Marchesi (Ref. 19) subsequently reported on three individual factor IX deficient patients in the post-operative period who developed a myocardial infarction associated with Konyne™ administration, elevation of serum fibrin split products after administration of Proplex™ but not after Konyne™, and pulmonary embolism with Konyne™ administration, respectively. Cutter Laboratories' experience is reported in Table VII (Ref. 20).

TABLE VI.—HEPATITIS B IN RECIPIENTS OF KONYNE™ COMPLEX

Patient	Factor deficiency (n=total number of infusions in all patients)	No. of infusions in patients with hepatitis B	Received other blood products	Severity of disease	Incubation period
1	II (2)				
2	VII (99)	1	No	NR	2.5 mo
3	IX (644)	3	No	NR	3 mo.
4		4	Previously.	Jaundice.	14 d.
5		7	Yes	SGOT, SPGT elevations	3 mo.
6		13	Yes	Jaundice.	2 mo.
7		4	Yes	NR	3 mo.
8		17	Yes	Jaundice.	2 week
9		10	Yes	Elevated SGOT, SGPT, no jaundice, possible subclinical case.	5 week
10	X (9)	3	Yes	SPGT 1,200, SGOT 1,800, No jaundice	2 mo

NR=Not reported.

TABLE VII.—PATIENTS WITH DEFICENCIES OF FACTORS II, VII, IX AND X

[Development of thrombosis or hepatitis]

Factor deficiency	Total No. patients	Total No. treatments	Total No. infusions	Number developing thrombosis	Number developing hepatitis B	Number receiving other blood products
II	1	1	2	0	0	0
VII	7	13	99	0	1	2
IX	78	126	644	1	[1]8	31
X	3	6	9	0	1	1

[1] Includes one possible subclinical case with elevated SGOT, SGPT, and no jaundice.

d. In addition to the above reactions, anaphylaxis has been reported (Refs. 8 and 9).

4. *Manufacturing analysis.* Manufacturing data are confidential, but a review indicated no reason to question the preparation of the product.

5. *Labeling recommendations.* a. It is recommended that the label should also contain:

(1) The amount of factors, II, VII, and X present by analysis if this material is recommended for treating these deficiencies.

(2) Thrombosis and hepatitis warnings.

(3) The amounts of sodium and potassium in the product in the final container.

b. The package circular should also:

(1) Give details of dosage and frequency of administration for each clotting factor deficiency to be treated.

(2) Include information about how to monitor effectiveness of treatment by employing clotting factor assays on the recipient's blood.

(3) Contain indications for use only in congenital deficiencies or urgent need for the rapid reversal of the effects of vitamin K antagonists.

(4) Include contraindications for use in newborns, liver disease, and surgical procedures in nondeficient patients, e.g., open heart surgery.

(5) State that after reconstitution, the material should be used within at least 4 hours.

6. *Recommendation.* It is recommended that the product be placed in Category I with the labeling recommendations as stated.

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Product Review

1. *Product.* Factor IX Complex (Human), (Factors II, VII, IX, and X) or Proplex ™ manufactured by Hyland Division of Travenol Laboratories is supplied as a 30-mL vial containing the lyophilized material. It is intended for intravenous administration through a filter after being reconstituted with 30 mL of sterile water for injection. Each bottle is assayed for factor II and factor IX activity or in vivo recoverable units. Other ingredients include heparin not to exceed one unit per Ml of reconsitituted product. No preservatives are used. The product should be stored between 2 and 3 °C.

2. *Effectiveness*—a. *Treatment of factor IX deficiency.* Eleven patients with factor IX deficiency were treated in one study with good hemostatic results in all (Ref. 1). The concentrates used had assay values of 5 units of factor II/mL, 17 units of factor IX/mL, and 20 units of factors VII and X/mL. This represented a purification of 30 times, 272 times, and 320 times, respectively. Protein concentration was 4.4 mg/mL.

Gilchrist (Ref. 2), on the basis of six infusions in four patients with factor IX deficiency, calculated that a dose of two units of factor IX/kg produced approximately one percent rise in factor IX plasma levels. The recovery values ranged from 17.3 to 33.6 percent with a residual factor IX activity of 40 to 59 percent at 24 hours posttransfusion. There were no changes in fibrinogen concentration of fibrinolytic parameters. In these early studies, protamine sulphate, 3 mg/vial of administered material was usually given to counteract the heparin in the concentrate.

Hyland (Ref. 8) provided data from 5 investigators on 42 infusions in 14 patients with factor IX deficiency. All patients treated had evidence of improvement, and there were no untoward reactions.

Levine (Ref. 3) reported 10 patients who used Proplex ™ in a self-therapy program. This material was reported to be effective in controlling hemorrhagic manifestations of spontaneous bleeding.

b. *Treatment of patients with liver disease.* Four patients with chronic liver disease with profuse bleeding from varices and/or hemorrhagic gastritis were given Proplex ™ infusions. Factor II levels were raised to normal with control of bleeding within 1 to 2 hours of the infusion (Ref. 4). One patient rebled and died several hours after completion of the infusion with evidence of accelerated disseminated intravascular coagulation (DIC). Half-life of factor II was 16 hours (recovery 60 percent), or

factor IX, 14 hours (recovery 42 percent), of factor VII and X, 7 hours (recovery 23 percent).

Cederbaum (Ref. 5) reported three patients with liver disease who developed DIC after infusion of Proplex ™ (two patients) and Konyne ™ (one patient).

c. *Other indications.* No data were found regarding effectiveness for treatment of factor II, VII, or X deficiencies, for treatment of patients with factor IX or factor VIII inhibitors, or for treatment of Coumarin overdosage.

3. *Safety*—a. *Hepatitis.* A single reference (Ref. 6) has reported hepatitis following Proplex ™ administration in a patient with factor X deficiency. (Also see the Factor IX (Human) Generic Statement.)

b. *Thrombosis.* A recent publication reported a lot of Proplex ™ documented to have resulted in thrombosis following infusion. On analysis, this material was found to contain activated factors IXa and Xa (Ref. 7). (Also see the factor IX (Human) Generic Statement regarding the thromboembotic potential of this product.)

4. *Recommendations.* a. Proplex ™ should be placed in category I, safe and effective, for the treatment of congenital factor IX deficiency.

b. Proplex ™ should be placed in Category IIIA for use in congenital and acquired deficiencies of factors II, VII, and X. A period of 3 years is recommended for the completion of the necessary studies to demonstrate effectiveness for these indications.

5. *Labeling recommendations.* a. It is recommended that the label should also contain:

(1) The amount of factors, II, VII, and X present by analysis if this material is recommended for treating these deficiencies.

(2) Thrombosis and hepatitis warnings.

(3) The amounts of sodium and potassium in the product in the final container.

b. The package circular should also:

(1) Give details of dosage and frequency of administration for each clotting factor deficiency to be treated.

(2) Include information about how to monitor effectiveness of treatment by employing clotting factor assays on the recipient's blood.

(3) Contain indications for use only in congenital deficiencies or urgent need for rapid reversal of effects of vitamin K antagonists.

(4) Include contraindications for use in newborns, liver disease, and surgical procedures in nondeficient patients, e.g., open heart surgery.

(5) State that after reconstitution, the material should be used within at least 4 hours.

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14. Fibrinogen (Human)

Generic Statement

1. *Introduction.* The end stage of coagulation is the conversion of the plasma protein fibrinogen to an insoluble polymeric gel called fibrin. The fibrinogen to fibrin conversion is a complex biochemical event catalyzed by thrombin, an enzyme generated in later stages of blood coagulation. Thrombin releases small polypeptides from fibrinogen leaving altered fibrinogen molecules, known as fibrin monomers, free to polymerize into the large fibrin units we recognize as blood clots. Deficiences or abnormalities of fibrinogen, whether hereditary or acquired, may lead to inadequate fibrin formation, a poor or absent blood clot, and abnormal bleeding.

Fibrinogen is synthesized in the liver, has a molecular weight of 340,000 plus or minus 20,000, and is normally present in plasma at a concentration of about 140–410 mg per dL of plasma (Ref. 1). Levels of fibrinogen increase in pregnancy, infection, cancer, granulomatous disease and many other pathological conditions (Ref. 1).

2. *Measurement of fibrinogen.* Fibrinogen can be measured gravimetrically, chemically, immunologically, or by approaches that utilize turbidity or light scattering (Ref. 2).

In some methods thrombin with or without calcium is added to plasma to generate clottable protein which is then measured and reported as fibrinogen (Ref. 3). Methods that utilize clot formation followed by washing, digestion, and chemical analysis of the clot are highly specific and accurate. Degradation products of fibrinogen and fibrin which are not active in the clot-forming process are not measured. These methods require meticulous attention to detail, accurate equipment, and take several hours before the answer is available, but give a reliable result. Digestion and chemical analysis of the clot can be replaced by carefully weighing the air-dried clot, but this is less accurate (Ref. 4).

The plasma clotting time after the addition of thrombin has been used as a repid screening method for afibrinogeninia (Ref. 5). More sensitive clotting time methods are also available and can be used to quantify fibrinogen in clinical settings. These have the advantage of simplicity and speed, but may not be sensitive to small changes in fibrinogen (Ref. 6).

Several turbidimetric methods are available for estimation of fibrinogen. These include salting out with ammonium sulfate (Ref. 7), sodium sulfite (Ref. 8), sodium sulfate (Ref. 9), or other chemicals. The methods are rapid and simple and in normal persons correlated well with more difficult methods. They are less reliable when the fibrinogen concentration is low or in sick persons where interfering substances may be present. In such cases a "grossly erroneous result" may be seen (Ref. 1). The turbidity that results when a dilute solution of fibrinogen reacts with thrombin can be measures easily and accurately to give a relatively sensitive, but specific, method for fibrinogen (Ref. 10).

Immunologic methods, usually based on the principle of radial immunodiffusion, are less accurate, detect fibrinogen or fibrin degradation products that have no clot-promoting activity, and usually require several days before an answer is available (Ref. 11).

Fibrinogen can be measured accurately, but many methods in common use are not completely reliable, and justification for therapeutic use of fibrinogen based on these techniques must be critically reviewed.

3. *Method of preparation.* Because fibrinogen is the least soluble of plasma proteins, it is isolated easily by relatively simple techniques. The plasma fractionation methods developed in World War II by E.J. Cohn and his associates allow the separation of plasma into multiple components (Ref. 12). Fraction I, separate at pH 7.2, temperature −3 °C with an 8 to 10 percent final concentration of ethanol, contains fibrinogen, factor VIII, and several other proteins (Ref. 12). Additional purification of the fibrinogen may be done with glycine (Ref. 13), ammonium sulfate (Ref. 14), or by other approaches. The preparation and purification of fibrinogen by these methods is relatively straightforward, and the assay of the final product can be accurately standardized.

4. *Clinical aspects of fibrinogen deficiency.* Fibrinogen is indicated for treatment of patients who are bleeding and have fibrinogen levels low enough to cause or contribute to the abnormal hemorrhage. Fibrinogen may also be used as prophylaxis in patients with abnormally low fibrinogen levels when a major stress to the blood coagulation system, e.g., surgery, is anticipated (Ref. 15). Because the fibrinogen to fibrin reaction is but one of a series of complex vascular and biochemical events leading to normal hemostasis, the fibrinogen level alone cannot be used to determine appropriate therapy. For the same reason, intravenous administration of fibrinogen is not, in most cases, adequate to reverse the hemorrhagic problem. In most cases, multiple abnormalities exist and simple infusion of fibrinogen will not produce normal coagulation.

The relationship of fibrinogen deficiency to abnormal bleeding is further confused by the presence, in many disease states, of abnormal activation of the fibrinolytic system. Thus, low fibrinogen may be a reflection of increased degradation of adequate amounts of fibrinogen; hence, attempts to correct the low level without simultaneously correcting the cause of the increased fibrinolytic activity may be deleterious rather than helpful (Ref. 16).

A similar dilemma occurs in the case of disseminated intravascular coagulation (DIC) (Ref. 17). The relatively recent konwledge that the hemorrhagic complications of many medical, surgical, and obstetrical disorders are related to widespread intravascular coagulation with consumption of coagulation factors, including fibrinogen, required that the treatmen of these problems be reevaluated. Disseminated intravascular coagulation can be acute or chronic and has been seen in such widely divergent states as gram-negative endotoximia, antigen-antibody reactions, abruptio placenta, amnotic fluid embolism, retained dead fetus in utero, hemolytic transfusion reaction, extracorporeal circulation, trauma, promyelocytic leukemia, thrombotic thrombocytopenic purpura, fat embolism, and poisonous snake bites. The laboratory findings vary in degree from patient to patient, but in all cases there is evidence of decreased levels of some coagulation factors, usually V, VIII, and fibrinogen; a low platelet count, and evidence of inceased fibrinolytic activity with the presence of circulating fibrinogen-fibrin degradation products. It is evident that many of the original theories of afibrinogenemia complicating various clinical conditions are better explained by widespread DIC. In this setting the previous emphasis on fibrinogen, both as an etiologic and therapeutic agent, requires reevaluation (Ref. 17).

5. *Levels needed for coagulation.* Because fibrinogen deficiency is frequently associated with other coagulation abnormalities, it is difficult to assign a critical fibrinogen value below which the risk of abnormal bleeding is increased. In one patient with hereditary afibrinogenemia, bleeding from a cut tongue stopped when the level reached 78 mg per dL (Ref. 18). Studies in this patient's plasma showed normal clot formation and retraction when fibrinogen values were as low as 50 mg per dL, although clot formation was slow. Other authors, usually reporting on bleeding in obstetrical patients, describe "unstable" clot formation when levels are in the range of 100 mg per dL but give no data (Ref. 19). Most review articles suggest that patients with plasma fibrinogen concentrations below 100 mg per dL have increased bleeding, but do not provide data to document the statement (Ref. 20).

6. *Specific indications.*—a. *Hereditary afibrinogenemia.* Approximately 60 patients with hereditary afibrinogenemia have been reported, all with fibrinogen levels of essentially zero. Clinical features include a hemorrhagic tendency with bleeding after slight trauma. Menses are normal and hemarthroses are rare. There is a long bleeding time and evidence of platelet dysfunction. The abnormalities, both laboratory and clinical, are corrected by small amounts of fibrinogen (Ref. 18).

b. *Hereditary hypofibrinogenemia.* Approximately 25 patients with a disorder marked by moderate reductions in fibrinogen level, with or without a mild hemorrhagic tendency, have been reported. These patients have not been well classified and at least one, on careful reinvestigation, has been shown to have hereditary dysfibrinogenemia (see next paragraph) rather than hypofibrinogenemia. There are no data to substantiate the need for, or efficacy of, fibrinogen in this condition (Ref. 21).

c. *Hereditary dysfibrinogenemia.* Since the first report, in 1958, of congenital abnormality marked by the production of defective fibrinogen at least 10 different varieties of hereditary dysfibrinogenemia have been reported. Some are associated with a bleeding disorder, and in at least three families recurrent thromboembolism has been a problem. The role of fibrinogen therapy is not defined (Ref. 21).

d. *Disseminated intravascular coagulation.* Widespread intravascular coagulation with consumption of circulating coagulation factors, utilization of platelets, and activation of the fibrinolytic mechanism has been established as the etiologic process for the coagulation abnormality seen in a whole host of different clinical conditions (Refs. 22 and 23), included among these are the serious coagulation abnormalities that accompany the obstetric complications of abruptio placenta, amniotic fluid embolism, retained dead fetus in utero, and induced abortion (Refs. 24, 25 and 26). The bleeding state in gram-negative septicemia, as well as that seen with many medical and surgical complications, is, in most cases, a manifestation of DIC (Ref. 27). Thus, nearly all hemorrhagic situations previously diagnosed and treated as acquired afibrinogenemia are now treated for consumption coagulapathy rather than by attempting to reconstitute the plasma level of fibrinogen with i.v. administration of concentrated fibrinogen. In general, the therapy of DIC is directed at the underlying pathologic problem rather than at attempts to replace deficient coagulation factors (Refs. 24, 25, 26, and 28). When the process is difficult or impossible to control, such as in metastatic cancer, heparin has been used (Refs. 29, 30, 31, and 32). The appropriate and judicious selection of anticoagulation (heparin), coagulation factor replacement, or therapy with fibrinolytic agents is a clinical decision of great difficulty. It is clear, however, that fibrinogen replacement is less important than had been previously believed, and that most patients do not need the infusion of fibrinogen concentrate.

(1) *Abruptio placenta.* The most frequent cause of severe bleeding during pregnancy is premature separation of the normally implanted placenta (Refs. 25, 26 and 33). The mechanism for the DIC is probably related to the destruction of the normal placental implantation with subsequent entry of decidual fragments, serum, and activated coagulation factors into the maternal circulation. Appropriate therapy includes prompt emptying of the uterus, treatment of shock, and blood transfusion (Ref. 24). Most patients do not require specific clotting factor replacement, and heparin, if indicated, is needed infrequently (Ref. 29). In those few cases where fibrinogen replacement is required, a dose of 4 g (Refs. 24 and 26) has been advised.

(2) *Intrauterine fetal death with prolonged retention of dead fetus.* In these cases these is slow continued absorption of thromboplastic materials from the retained dead fetus leading to chronic activation of the coagulation system in vivo. This produces a mild chronic DIC usually requiring about 5 weeks for full development. Appropriate therapy includes frequent monitoring by coagulation tests, including fibrinogen, along with prompt evacuation of the uterine contents as soon as significant abnormalities become evident. Intravenous administration of fibrinogen is rarely, if ever, required (Refs. 24 and 26).

(3) *Amniotic fluid embolism.* The entrance of amniotic fluid into the maternal circulation, usually about the time of delivery, can produce a sudden and catastrophic event marked by respiratory distress, cyanosis, shock, and acute DIC. In most cases the onset is sudden and death occurs within minutes. At times the shock develops more slowly, and widespread bleeding is noted before death (Refs. 25 and 26). Since amniotic fluid is rich in thromboplastic materials, the development of widespread consumption coagulopathy is not surprising (Ref. 26). These patients require immediate and massive treatment for shock, transfusion to replace blood loss, and fibrinogen replacement (Refs. 25 and 26). Four grams of fibrinogen have been recommended (Ref. 24).

(4) *Miscellaneous conditions.* Massive trauma, burns, incompatible transfusions, septicemia (particularly with gram-negative organisms), promyelocytic leukemia, carcinoma, hepatitis, virus infections and, indeed, almost any serious medical or surgical illness may, at times, be associated with DIC. Therapy in these cases varies, but the use of intravenous fibrinogen is not a major therapeutic modality (Refs. 17 and 34).

7. Risks and complications. Fibrinogen is prepared from the plasma of large numbers of donors. Because of this and because fibrinogen will not tolerate the 10 hours of heating at 60° C used to inactivate the hepatitis virus in other blood fractions, fibrinogen administration has been associated with a significant risk of transmitting viral hepatitis (Ref. 35). The actual current risk is, however, almost impossible to evaluate since:

a. Most studies do not have a careful followup of recipients.

b. In almost all cases, recipients of fibrinogen have also received other blood products.

c. There are no reports of the hepatitis incidence after fibrinogen prepared from donor plasma that is negative when tested for HBsAg by third generation methods. Since this is now a manufacturing requirement, reports from the 1950's and 1960's may not reflect the current risk.

Bearing these limitations in mind, the reported incidence of postfibrinogen hepatitis has ranged from 1.7 percent to 55 percent. The two most recent reports (Refs. 36 and 37), both from Michigan, give divergent hepatitis rates and also illustrate the difficulty of evaluating the published data. In one study (Ref. 36), the manufacturer received reports on 649 patients who had received an average of 2.2 units of Fibrinogen (Human) per patient. Of these, 452 were followed for 6 months. One case of hepatitis (1.7 percent incidence) was reported in the 50 recipients who received Fibrinogen (Human) alone, while 34 cases (8.6 percent incidence) were reported in the 394 patients who had also received an average of 7.0 units of whole blood. All the plasma from which the Fibrinogen (Human) had been prepared was from volunteer donors, but had not been tested for HBsAg. In addition, there was no effort to follow recipients, hence the reported incidence must be considered as the absolute minimum. A second study (Ref. 37), also from Michigan, where recipients were "followed at close intervals by careful physical examinations and laboratory tests," reported the incidence of hepatitis to be 55.5 percent in the 9 patients who received Fibrinogen (Human) and survived longer than 7 days. Unfortunately, the fibrinogen was partly from commercial donors and partly from volunteers. In addition, of course, the data were collected before HBsAg testing was required.

There are no data about the hepatitis risk from Fibrinogen (Human) prepared from HBsAg negative donors, but it is known that such donors may transmit hepatitis and that with current processing methods HBsAg is found in fibrinogen fraction (Refs. 38, 39 and 40). Thus it is probable, but not proven, that large plasma pools, even though prepared from HBsAg negative starting materials, will be infectious. There are no data for Fibrinogen (Human), but other fractions, such as Antihemophilic Factor (Human) and Factor IX Complex (Human), have been more widely used and studied. Since pool sizes and general processing methods are similar for all of these unheated fractions, it is reasonable to assume that the risk of hepatitis transmission is also similar and that risk is from 36 to 75 percent in recipients who have had little or no other contact with blood products (Refs. 41 through 45). Again, these data were collected before the requirement for HBsAg testing on each donor unit of starting material. This fact notwithstanding, the high hepatitis risk of pooled unheated material is evident. Elimination of HBsAg-positive donors will lower, but not eliminate, this risk, and lacking more current data the wisest course is to accept previous estimates of the hepatitis risk for fibrinogen of about 25 percent.

At least one death has been ascribed to diffuse intravascular thrombosis after fibrinogen administration in a surgical patient (Ref. 46), and several cases of severe pulmonary embolism have been seen (Refs. 47 and 48).

Coomb's positive hemolytic anemia, probably related to the presence of anti-A and anti-B antibodies in the product, has been seen after large doses of fibrinogen (Ref. 49). This complication has been reported only in patients with hemophilia who were treated with large doses of fibrinogen rich in Factor VIII. Although fibrinogen is no longer considered appropriate therapy for hemophilia, similar doses of fibrinogen administered for other indications could have the same effect.

Various side effects including hypotension, tachycardia, nausea, vomiting, paresthesias, urticaria, fever, chills, and headache have been noted occasionally but are difficult to evaluate since, in each case, the product was being administered to an extremely sick patient who had multiple complicating factors (Refs. 50 through 52).

8. Other sources of fibrinogen. Fibrinogen (Human) is a licensed product currently recommended for the indications stated above. Fibrinogen replacement, however, can be

accomplished with other blood components or fractions. Cryoprecipitated Antihemophilic Factor (Human), (cryoprecipitate), while usually prepared as a source of Factor VIII for the treatment of hemophilia, is also an excellent source of fibrinogen, since each bag of cryoprecipitate contains approximately 250 mg of fibrinogen (Refs. 53 and 54). Cryoprecipitate has been used in patients who were candidates for fibrinogen replacement (Refs. 55 and 56). The hepatitis risk is unknown, but is probably equal to or less than that associated with whole blood transfusion (Ref. 57).

9. *Recommendations.* Recent evidence including a multitude of well documented studies, has established disseminated intravascular coagulation as the major, or perhaps the only, etiological factor in the development of acquired hypofibrinogenemia. As such, therapy is appropriately directed at the underlying disease. Heparin is sometimes used, and fibrinogen replacement is almost always unnecessary. In those few patients with acquired hypofibrinogenemia or in the rare patient with hereditary a-, hypo-, or dysfibrinogenemia, cryoprecipitate is an adequate source of fibrinogen replacement.

The hepatitis risk of cryoprecipitate relative to Fibrinogen (Human) is unknown and, because of the relative infrequency of patients who require fibrinogen replacement, is not amenable to appropriate study. Thus, there are not and will not be, scientifically sound data from which to decide about the risk of hepatitis from Fibrinogen (Human). Traditionally the hepatitis risk from pooled products that cannot be heat treated is high. The exclusive use of HBsAg negative donors reduces, but does not eliminate, this risk. It is well known that even small amounts of virus can contaminate large pools and that the risk of infection increases as pool size increases. Thus, it seems logical to restrict therapy to components, fractions, and derivatives that supply the required factor with the lowest possible risk of exposure to potentially infectious materials. In the case of fibrinogen deficiency, the desired treatment can be achieved with cryoprecipitate. Even the most generous dosage schedules will expose the patient to relatively few donors. The substitution of cryoprecipitate, Single Donor Plasma (Human), or Single Donor Plasma (Human) Fresh Frozen for Fibrinogen (Human) in those few cases where fibrinogen is indicated will decrease donor exposure and should diminish hepatitis risk. Lacking definitive data about relative risks from cryoprecipitate and fibrinogen, the Panel recommends that cryoprecipitate be used as a source of fibrinogen in the few clinical situations when such therapy is indicated. The Panel recommends that Fibrinogen (Human) as presently prepared be placed in Category II.

The recommendations of the Panel were forwarded to the Commissioner of Food and Drugs following the January 14–15, 1977 meeting of the Panel, and all licenses for Fibrinogen (Human) were subsequently revoked as of December 7, 1977, as reported in the **Federal Register** of January 6, 1978 (43 FR 1131).

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15. Fibrinolysin (Human)

Generic Statement

The coagulation system is complex and rigidly modulated. The "other side" of this system, the clot-lysing system, is apparently nearly as complex and as rigidly controlled, but is less well understood. Clinically, it is not less important. The ability to manipulate this system with certain agents is of potential therapeutic significance. References 1 through 3 support the basic discussion of the fibrinolytic system.

Plasminogen is a protein normally found in plasma. It is a single-chain beta globulin and can be purified from Cohn Fraction III. Its normal concentration is 200 micrograms/mL. Lower levels are found in infants and patients with cirrhosis. Higher levels are found in patients with infection, malignancies, or after injury. Two general forms have been identified, one with glutmatic acid in the N-terminal position (Glu-plasminogen) and one with lysine (Lys-plasminogen). The Glu form is slightly larger and is probably the natural circulating form. The Lys form, molecular weight 82,000–83,000, is probably a partially digested form. It has a shorter half-life after infusion into man (0.8 vs. 2–2.5 days), and is more quickly activated and is more readily adsorbed to fibrin. This latter property may have therapeutic implications (Ref. 4). Plasminogen is rapidly synthesized, with plasma levels rising from none to normal within 12 to 24 hours after it has been depleted by infusion of an activator.

Plasminogen as it circulates is inactive. When it reacts with plasminogen activator, it is converted to plasmin, which lyses fibrin clots.

Plasminogen activators or precursors of activators are found in blood, many tissues (not liver), urine, saliva, and semen. There are high concentrations in vascular endothelium, more in veins than in arteries. The concentrations of activators in blood increase rapidly after vigorous exercise, emotional stress, injury, adrenergic stimulation, and following venous occulusion or ischemia of tissue or the infusion of various substances. The vascular endothelium may be the site of release of plasminogen activator into the circulation in response to many of these stimuli. Activated Factor XII promotes the formation of a plasminogen activator that may be different from the above. After injury or operation, the early rise in the levels of plasminogen activators in the blood is followed by at least several days of lower-than-normal levels.

The best-characterized plasminogen activator is urokinase. It is found in normal human urine and probably produced by renal parenchymal cells. Urokinase is a single chain beta globulin of molecular weight 54,000. It is not antigenic to humans. Many bacteria produce plasminogen activators, the best known of which is streptokinase. It is produced by beta-hemolytic Streptococci and is a protein with the mobility an *alpha*-2-globulin, and a molecular weight of 47,000. It is strongly antigenic in man.

When plasminogen and a plasminogen activator interact, plasmin is produced. The best defined reaction of this type is with urokinase. The product, plasmin, which forms has one heavy and one light chain linked by a single disulfide bridge. The light chain is the site of plasmin enzymatic activity. With streptokinase the reaction is less well understood, but a double-chain plasmin molecule is also formed. Streptokinase alone has no proteolytic activity. It combines with both

plasminogen and plasmin and undergoes structural rearrangements. The resulting complex acts as the "activator," and the streptokinase is partially fragmented in the process.

Plasmin is a serine protease, like many other enzymes formed from precursors. Like most such enzymes, it is fairly nonspecific and can digest a wide variety of substrates. In the coagulation system these include fibrin, fibrinogen, Factors II, V, VIII, and possibly VII. It also activates Factor XII and thereby can activate the coagulation, complement, and kinin systems (Ref. 35).

Such a powerful and nonspecific system must have a number of control mechanisms. Some of these have been identified. There are inhibitors to both plasmin and plasminogen activators. The anti-activators are found normally in the circulation, are probably *alpha*-2-globulins of molecular weight 75,000–80,000, and are fairly nonspecific (Ref. 37). Antibodies to streptokinase are specific anti-activators. These effectively neutralize infused streptokinase. They are found in almost all normal humans as the results of prior exposure ot streptococcal organisms. The level in the circulation varies widely, and will rise sharply in response to exposure to streptokinase through infection or infusion.

There are a rather large number of anti-plasmins. A fast-reacting inhibitor, highly specific for plasmin, may be the most important (Ref. 36). *Alpha*-2-macroglobulins are thought to be the principal secondary forms; they react rapidly but reversibly with plasmin in the circulation. Alpha-1-antitrypsin is also an anti-plasmin that reacts slowly but irreversibly. Other, perhaps less important, anti-plasmins are the anti-thrombin III-heparin complex, C 1 inactivator, inter-*alpha*-trypsin inhibitor, an inhibitor found in platelets, and inhibitors in granulocytes, arterial walls, and the placenta. The liver seems to be an important site of production of some anti-plasmins, and is also a site of inactivation of circulating activators. Patients with impaired hepatic function seem more prone to develop "out of control" fibrinolytic responses to various stimuli.

The products of the interaction of plasmin with both fibrinogen and fibrin can interfere with fibrin polymerization and can interfere with platelet function and therefore promote the development of clinical bleeding disorders (Ref. 1). Again the liver, or at least the reticuloendothelial system, seems to be an important site of clearance of fibrin or fibrinogen fragments from the

It is still not clear how the activity of plasmin, once formed, is limited to the area of need. There are a number of theories or hypotheses, but no real proof. The most widely accepted theory holds that plasminogen is adsorbed to fibrin. Plasminogen activator diffuses from the circulation into the clot, where plasmin, inhibitors are absent (Ref. 5). An alternate theory holds that plasmin-anti-plasim dissociates when in contact with fibrin and the plasmin becomes more tightly bound to the fibrin (Ref. 6). Another proposal suggests that plasminogen activators are selectively bound to fibrin (Ref. 7). In the background is preliminary evidence that fibrinolysis, not mediated by plasmin, may be clinically important.

There are marked differences among animal species in the activation and function of the plasminogen-plasmin system. Many laboratory animals respond to streptokinase with little or no activation of plasminogen. Man responds consistently by activating plasminogen when the proper dose is used. This has made evaluation of these agents, especially those containing streptokinase, almost impossible in controlled laboratory experiments and has required careful clinical study in man.

Product Review

1. *Description.* The Merck Sharp and Dohme Fibrinolysin (Human) "Thrombolysin™" product consists of a mixture of streptokinase (SK) and human plasminogen. Plasminogen is prepared from Cohn's Fraction III of human plasma. SK is prepared from the supernatant of cultures of streptococcus organisms. SK is added to the plasminogen, producing a mixture of SK, SK-plasminogen, SK-plasmin, plasmin, and other reaction products. It is not known if the plasmin is in the glutamic acid or lysine form. One vial of the substance containing "50,000 fibrinolytic units," contains about 40 units of plasmin and 20,000 units of SK. The manufacturer's "fibrinolytic units" relate to the ability of the active product to lyse a standard fibrin clot under certain conditions and in a specified period of time. The more commonly used units of measurement are determined by the ability of the active enzyme to hydrolyze casein (proteolytic activity) and the ability to convert additional plasminogen to plasmin as measured by resulting hydrolysis of casein (activator units). This review will refer to the latter two methods of measurement.

No known method of measurement relates simply and directly to human in vivo effects of this product. Most normal inhibitory units per mL of plasma, or about 10,000 to 20,000 total circulating plasmin-inhibitory units. This obviously is vastly greater than the amount of plasmin usually infused with this product. Some investigators claim that the entire effect of this material is due to the in vivo activation of the recipient's plasminogen to plasmin by the streptokinase component, and that the preformed plasmin of the product is inconsequential. Most available evidence favors such an interpretation. There is a countervailing hypothesis that the inhibitor-plasmin complex formed almost immediately after infusion remains circulating for a time and is readily reversible. Fibrin forms a stronger bond with plasmin and is irreversible. Therefore, plasmin inactivated by inhibitor remains largely available by dissociation and active in in vivo fibrinolysis. There is little direct evidence to support this hypothesis; its main proponent has claimed therapeutic success with an SK-plasmin preparation from which the activator "had been removed" in a study involving five patients but without adequate controls (Ref. 8).

Normal individuals have about 3.8 plasminogen units per mL of plasma, so that the amount of activator (SK) infused with this product is potentially significant in activating the recipient's own plasminogen. Essentially all patients, however, have detectable levels of antistreptokinase, varying from 10,000 to 6 million circulating anti-SK units. Thus, even apart from the effects of plasmin inhibitors, there will be a wide range of effects from infusion of the SK of this product into a randomly selected population because of varying inactivation of this most important therapeutic component. Various investigators have proposed measuring anti-SK levels on all recipients and adjusting dosage accordingly, or titrating the dose to achieve a predetermined effect on a suitable laboratory test, such as prothrombin time, fibrinogen level, plasma recalcification time, or other tests, or both. Certainly, one of the major problems in evaluating the reported clinical experience is the lack of evidence that a physiologic effect upon the plasmin system was obtained in all patients with the dose used. Very few of the available studies contain satisfactory data in this regard.

The commercial product is supplied in dried form and must be mixed just before use. It should be kept cool during administration. At 4 °C loss of activity rarely exceeds 10 percent in 8 hours, but

reported as rapidly lost (up to 25 percent in 15 minutes) in freshly reconstituted products. The manufacturer adds a stabilizer and states that, when reconstituted, the product loses 10 percent of its activity in 2 hours and 25 percent in 4 hours at room temperature. It is not clear from clinical studies whether proper precautions were taken to guard against loss of activity.

2. *Safety.* The most recent reports of clinical use indicate a much lower incidence of febrile reactions than were common when the material was first marketed. This is now a fairly minor problem despite the known pre-immunization of almost every potential recipient to SK. This seems to have followed improvements in the purification of SK.

Anaphylaxis has been rare. The only reactions reported occurred following repeated parenteral administration of SK (Ref. 8). It is clear that careful consideration must be given before the repeated parenteral administration of a product containing SK. The need should be great, the circulating levels of anti-SK probably should be measured and an attempt be made to eliminate or lower the level of circulating antibody by administration of graded doses of SK before attempting a full therapeutic plasminogen-activating exposure.

Four instances of hepatitis were reported in a single series of patients early in the use of this product (Ref. 9). There have been no reported instances in the past 15 years. The product is heated at 60 °C for ten hours before release.

Hemorrhagic complications have been variously reported (Refs. 8 through 13). In general, the development of hemorrhage seems to parallel the induction of an active fibrinolytic state as detected by various laboratory tests; it occurs most easily at sites of recent injury, including venapuncture, and seems more common when anticoagulant drugs are used simultaneously (Refs. 8 and 12).

There is no generally agreed-upon method for monitoring the effects of this product on the recipient's coagulation system. Direct measurement of the components and activities of the plasminogen-plasmin system is technically demanding and is not routinely available. The "indirect" effects of the material have led various investigators to propose serially measuring bleeding time, whole blood-clotting time, thrombinclotting time, prothrombin time, fibrinogen concentration, thromboplastin generation, and plasma recalcification time among others. Fibrinogen concentration and one-stage prothrombin times seem the most consistently affected. Lengthening of the thrombin time or the prothrombin time seems to provide indirect evidence of the desired therapeutic effect at a state before severe effects which may result in bleeding are produced. Serious bleeding has followed the unintended induction of significant hypofibrinogenimia and digestion of other coagulation factors by plasmin with marked prolongation of the one-stage prothrombin time. Such patients have exhibited extensive activation of the plasminogin-plasminogen-plasmin system.

Other reported side effects include nausea, vomiting, abdominal pain, and, rarely, skin reactions or hypotension.

3. *Effectiveness.* The ability to dissolve clots intravascularly seems to vary with the age of the clot (easier when fresh), the circulating levels of fibrinogen and lipids (higher levels seem to impair efficacy) the status of inhibitors and anti-SK antibodies, hepatic function, perhaps the composition of the clot, and certainly the underlying condition(s) causing the intravascular thrombosis (Refs. 1 through 3 and 8). The benefit or harm to the patient from lysing an arterial clot, if such occurs, depends on the reversibility of tissue damage beyond the area of occlusion and the chances for harmful hemorrhage in the reperfused area. With venous clots, there is the theoretical danger of increasing the chances of pulmonary embolization by partially dissolving and "freeing up" thrombi. This seems not to occur.

The greatest problem in evaluating efficacy is the lack of properly controlled clinical trials, and the minimal value of studies on laboratory animals treated with this product. In addition, details of dosage and of handling the material and documentation of an effect on the proteolytic system are often lacking, as is angiographic evidence of the presence of thrombi before treatment and the dissolution of thrombi during treatment. The result is overall poor documentation of claimed benefits. Further, this product should be considered with closely related products, SK alone and urokinase from the point of view of safety and effectiveness.

4. *Specific clinical uses*—a. *Arterial thrombosis and embolization.* There were many reports of the use of this product alone or in conjunction with surgical intervention in the treatment of arterial thrombosis and embolization, mostly in the early 1960's (Refs. 14 through 17). By current standards, none of these studies were properly controlled. Often, there are underlying mechanical problems when an arterial thrombus develops, and the reports available are rather anecdotal and unconvincing. It is unlikely that surgery can be avoided by the use of this material, and there is no evidence that the outcome is improved. Techniques of vascular surgery such as better angiography, noninvasive flow measurements, better suture material, balloon catheterization, and extraction of clots have improved significantly since the earlier clinical studies and supplemental fibrinolysin now seems to be used rarely.

There is one important possible exception. Recent reports on the use of SK locally in structures used for vascular access for hemodialysis (shunts, fistulas, heterografts) have been impressive in that a number of patients who would otherwise have required surgical revision were carried for months after clotting of the devices had occurred and other usual means of restoring flow had failed. This is a small but important group of patients because these devices are literally life sustaining. Much of the time, thrombosis signifies an underlying mechanical problem which must be corrected. Some of the time local fibrinolysis seems to be effective. Since the material is used locally, systemic effects are minimal, making the technique even more attractive. Better-controlled studies are needed to indicate the proportion of such patients that can indeed be helped by this approach, but the reported experience to date is encouraging.

b. *Myocardial infarction.* The theoretical problems encountered when evaluating the effect of fibrinolysis in myocardial infarction are the uncertainty about how many myocardial infarctions are due to fibrin clots in the coronary arteries, and the problems of attaining reperfusion after infarction has occurred. The practical problems are those of establishing proper controls and demonstrating the presence of clots before treatment and their absence after treatment. Many reported studies are too small to draw conclusions or are uncontrolled. One very small study claimed less rise in SGOT in seven treated patients versus five controls (Ref. 18). Another claimed faster restoration toward normal of EKG abnormalities in 15 treated versus 40 historical "controls" (Ref. 19). Another series of papers dealing with a continuing series of patients claimed lower mortality in treated versus untreated patients, but the series was only partially controlled, and if the seemingly early uncontrolled group is removed, the differences disappear (Ref.

20). Three sizeable controlled studies with a total of 178 treated patients versus 182 control patients showed no differences in mortality (Refs. 9, 12 and 17).

c. *Cerebrovascular accidents.* Use of Fibrinolysis in ischemic strokes has not been shown to be beneficial in the best reported study to date (Ref. 21). This was a prospective double-blind study without angiography involving 20 treated patients and 20 control patients. In other smaller uncontrolled series the authors speculated as to benefit, but unconvincingly. The brain is one area where restoring blood flow rapidly to irreversibly damaged tissue undergoing necrosis conceivably could do more harm than good.

d. *Deep venous thrombosis.* This is one of the most troublesome therapeutic problems to evaluate. The recent resurgence of interest and investigation in this disease has clearly shown the inadequacy of relying on clinical criteria for either diagnosing the presence of clots or of detecting their dissolution. None of the early studies were properly documented by venography by modern standards, and very few were properly controlled even for clinical signs and symptoms (Refs. 8, 10, 11 and 17). Some compared such nebulous criteria as pain, disability, and duration of hospital stay with historical controls. In one of the few studies using simultaneous controls and three treatment groups, fibrinolysin, heparin, and rest and elevation only, the group treated with heparin seemed to fare better than the other two, which were indistinguishable from each other (Ref. 22). There was no angiographic documentation, and "superficial" phlebitis was included as were patients with underlying malignancy; this study is thus of doubtful merit. A more recent study indicated earlier lysis of deep venous clots with the infusion of SK and plasminogen separately (Ref. 4). This is not the same material as Thrombolysin™, but it is close.

The other problem with evaluating the treatment of deep vein thrombosis is in the selection of the criteria of success. Most pulmonary emboli result from clinically inapparent clots. The real problem with deep vein thrombosis is in the late sequelae. No study of fibrinolysis has adequately addressed this problem. The experience with ileofemoral thrombectomy indicates that early apparent benefit can disappear when late sequelae are evaluated at 5 to 10 years following the event (Ref. 23). Proof of efficacy in this disease will require a controlled study with angiographic documentation, and prolonged followup with double-blind evaluation of late sequelae.

e. *Pulmonary embolism.* The recent controlled trials with various fibrinolytic agents show that large pulmonary emboli can be lysed more rapidly when such agents are used (Refs. 24 and 25). Spontaneous lysis occurs at a significant rate also, so use of such agents should probably be limited to special circumstances, such as persisting hemodynamic changes or repeated embolization. One of the largest groups in which such criteria are likely to apply are patients soon after accidental or operative injury. This is the group in which the risks of significant hemorrhage are quite high following induced fibrinolysis. A better definition of benefit and risk is needed in such a group of patients before reliable conclusions can be drawn. For those patients without contraindications, use of lytic agents in the above circumstances seems warranted. These comments are based on the assumption that Thrombolysin™ is functionally similar to streptokinase, which was studied in some of these trials. Thrombolysin™ was not itself evaluated in these studies. Reports of the treatment of pulmonary emboli with Fibrinolysis (Human) are seriously lacking in either controls or angiographic documentation or both (Refs. 9, 17, 26 and 27).

f. *Thrombosis of the retinal artery or veins.* There seem to be only two reported instances in which there was significant return of vision following treatment of retinal arterial thrombosis with thrombolysin (Ref. 28). The oxygen demands of the retina are so high and the arterial supply so vulnerable, that it seems unlikely that a complete thrombotic occlusion could be lysed early enough to allow function to recover. It is not clear how often partial occlusions occur. There are more encouraging reports of improvement of vision after the treatment of occlusion of the central retinal veins, but none of these reports have presented proper controls (Refs. 10, 11 and 29).

g. *Traumatic hyphema.* Instillation of fibrinolysin into the eye to remove post-traumatic clots was reported with some frequency but with no controls. The closest to a controlled study seemed to show equal benefits from conservative treatment (Ref. 30). This use of fibrinolysins seems to have ceased in recent years and cannot be recommended.

h. *Priapism.* There have been several reports of the use of systemic and/or local fibrinolysin for the treatment of priapism, all without adequate controls (Refs. 10, 31 and 32). This is a disorder with a wide range of causes, some unlikely to be helped by fibrinolysin. Again, this does not seem to be a currently practiced method of treatment.

i. *Respiratory distress of the newborn.* One rather striking study showed a sharply increased mortality from the use of systemic fibrinolysin for the treatment of respiratory distress of the newborn in which 1 of 6 controls died versus 5 of 5 treated (Ref. 33). Autopsies yielded no clues as to the cause, but this experience suggests that this material should not be used parenterally in premature infants for any reason. Others have used it by pulmonary insufflation in respiratory distress of the newborn without such adverse results but also without apparent benefit.

j. *Intraperitoneal adhesions.* A single study in rats suggested fewer intraperitoneal adhesions after the use of intraperitoneal fibrinolysin (Ref. 34). The experimental model and the problems with extrapolating to another species makes this observation of uncertain significance for man. No clinical studies have been reported.

5. *Labeling.* The label available for review uses the manufacturer's dosage units, which differ from those commonly used in the literature. This can be misleading. The discussion in the labeling of published reports on the use of this product in various conditions does not refer to or properly emphasize some of the negative clinical trials, especially in patients with myocardial infarction and cerebrovascular accidents. The only indications listed for use are "phlebothrombosis, thrombophlebitis, pulmonary embolism, and thrombosis of arteries with exclusion of thrombosis of coronary arteries and cerebral arteries." The limitations of fibrinolytic therapy in such conditions, discussed in this review, are barely or not at all mentioned. There is perhaps an undue impression of accuracy of dosage, but in view of the uncertainty about proper dosage and monitoring, this is not an area in which definite statements can or should be made. The precautions do not list concurrent anticoagulation as probably increasing the risk of hemorrhage; this point seems well enough established to be included in the labeling.

6. *Summary.* Fibrinolysin (Human) was first evaluated clinically several years before the current resurgence of interest in the study of thromboembolic disease. As a result, its evaluation for effectiveness has been inadequate by modern standards. Improper or no controls and lack of angiographic

documentation are the two most serious and common defects. In many published studies, evaluation of hemostatic function was either not done or was inadequate to assess whether or not a fibrinolytic state had been induced. This is a particularly important point with a product containing streptokinase because of the variable level of inactivating antibodies in the human population. The extremely limited relevant data that can be obtained in animals with this product make careful human clinical studies mandatory.

The most likely clinical uses of value are for pulmonary embolism, deep venous thrombosis, and clotted vascular access devices in patients undergoing hemodialysis. The limitations and problems for these uses have been discussed. For all three uses, most of the pertinent data have been derived from studies using urokinase or streptokinase alone. One of the main problems the Panel faced was in deciding to what extent these data on SK could be applied to Thrombolysin™. It seems likely that most of them are applicable, but this is in fact a different product. There is little reason to expect much functional difference between these products, and the available data do indicate that Fibrinolysin (Human) can activate the plasmin system in human recipients. The Panel's first decision, then, was to treat this product in a manner similar to urokinase and streptokinase, and to allow market forces to decide its utilization, even though it seemed logical that if both a plasminogen activator and plasminogen itself were needed in specific circumstances, these should be provided separately in the doses indicated. The regulations in 21 CFR 601.25(d)(4) appear to require specific data supporting the need for each component of such a combination. The recommendation of the Panel follows the regulations, since the requirement for the plasminogen component cannot be documented by present data.

7. *Recommendations.* a. Fibrinolysin (Human) should be placed in Category IIIA, that is, to remain on the market pending proof of effectiveness, but requiring that such proof be presented to retain permanent licensure. A period of 3 years should be allowed for the recommended studies demonstrating the contribution of each component to the labeled indications.

b. During continued marketing, the only indications for which there is reasonable supportive evidence at present are for use in the treatment of pulmonary embolism, deep venous thrombosis, and clotted vascular access devices. Labeling for any of these indications should reflect the problems discussed in this review. Labeling should indicate that spontaneous lysis occurs with both pulmonary emboli and deep venous thrombi, that fibrinolytic agents appear to accelerate the early phases only, that there is no proof that late sequelae are lessened in patients with deep venous thrombosis, and that underlying mechanical factors are often the cause of thrombosis in vascular access devices.

c. All fibrinolytic agents should be rereviewed at a later date by a single panel of experts. Separate reviews of urokinase and streptokinase on one hand and Fibrinolysin (Human) on the other is artificial, duplicative, and could result in unfair inconsistencies.

d. The material should be named "Fibrinolysin."

8. *Other research.* The following are areas for which further research is recommended:

a. There is a great need for a practical laboratory test which can be used to monitor therapy with fibrinolytic agents.

b. The fibrinolytic system itself needs considerable further elucidation.

c. Plasminogen activators are needed which are effective, nonantigenic, and less expensive than urokinase as now produced.

d. The breakdown products of fibrin and fibrinogen need to be further defined, their effects and dynamics studied, and methods for their detection need to developed which will distinguish the various stages of fibrinolytic events in patients.

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16. Rh_o(D) Immune Globulin (Human)

Generic Statement

1. *Introduction.* Hemolytic disease of the newborn due to anti-Rh_o(D) was a significant problem prior to the advent of immunoprophylaxis in 1968. Milestones in the understanding and management of this problem include the discovery of the Rh_o(D) antigen, elucidation of the pathogenesis of the disease, the development of exchange transfusion in the neonate, the development of intrauterine diagnosis by amniocentesis and intrauterine transfusions. Despite these formidable achievements, it was estimated that in 1968, 2.7 deaths per 10,000 live births occurred in the United States as a result of this condition (Ref. 1).

The pathogenesis of Rh hemolytic disease of the newborn is well known. In a primigravid Rh-negative woman who has not been exposed previously to Rh-positive blood, primary immunization occurs most frequently during labor and delivery, particularly during the third stage of labor, when Rh-positive fetal red blood cells (RBC) cross the placenta and enter the maternal circulation in substantial numbers. The risk of immunization is related to the magnitude of feto-maternal hemorrhage: In the presence of ABO compatibility between mother and fetus, the risk is 3 percent when less than 0.1 mL of the fetal RBC are present in the mother, is 25 percent when 0.25 to 1 mL are present, and is 65 percent when more than 5 mL are present. Perhaps 75 percent of delivering women have less than a 0.1 mL feto-maternal hemorrhage while only 0.3 percent have feto-maternal hemorrhage of greater than 15 mL. Thus, the overall risk of Rh immunization in ABO compatible pregnancies is 13 to 15 percent per Rh incompatible pregnancy. In ABO-incompatible pregnancies the risk is 2 to 3 percent (Ref. 2).

A major development in the elimination of this problem occurred in the late 1960's when it was demonstrated by a number of investigators that active immunization of Rh-negative mothers carrying Rh-positive fetuses could be prevented with the administration to the mother of an intramuscular injection of Rh_o immune globulin within 72 hours after delivery (Refs. 3 through 6). Using the standard dose of 300 micrograms of Rh antibody, there has been a 90-percent reduction of the immunization rate from about 15 percent to about 1.5 percent (Refs. 2 and 7). The reasons for failure to achieve complete prophylaxis are not entirely clear and may reflect, in part, a lack of clear understanding of the mechanism by which prophylactic effect is achieved. Failures may result from a number of factors:

a. Immunization may have already occurred during pregnancy. Fetomaternal hemorrhage has been noted as early as during the early portion of the second trimester and increases in size and frequency as gestation proceeds (Ref. 2). Thus, some women may be immunized early in pregnancy. In recognition of this, some investigators have administered Rh_o immune globulin during pregnancy in either single dose (300 μg) at 34 weeks gestation or twice at 28 and 34 weeks gestation. The evidence to date suggests that as many as 65 to 90 percent of all prophylaxis failures may be eliminated by antepartum administration of Rh_o immune globulin (Refs. 8 and 9). These studies require demonstration that immunization has not occurred by evaluation subsequent pregnancies. Infants born after antenatal treatment show no evidence of increased red cell destruction resulting from the passive antibody administration to the mother, although many manifest a positive direct antiglobulin test of their red cells.

b. A large feto-maternal bleed may have occurred, and the standard 300 microgram dose is therefore inadequate. Studies indicate that 300 micrograms of Rh antibody are sufficient to prevent immunization when up to 15 mL of Rh-positive red cells have been infused into Rh-negative subjects (Refs. 10 and 11). As only 0.3 percent of all pregnancies will have feto-maternal bleeds of greater than 15 mL (Ref. 2), this factor cannot account for more than than a small number of all the Rh_o immune globulin prophylactic failures. In most pregnancies, the dose of anti-Rh_o is probably excessive.

c. It is possible that the intramuscular route of administration may not assure adequate uptake of antibody into the circulation. While there is no direct evidence on this point, European investigators have been using intravenous administration of a preparation prepared especially for intravenous use (Ref. 12). The World Health Organization has recommended that only half the usual dose is necessary if the intravenous route is used.

d. Rh-negative women born of Rh-positive mothers may have been actively immunized by a materno-fetal bleed when they were born ("grandmother theory"). When these women deliver an Rh-positive fetus, Rh_o immune globulin would not be effective because of their prior immunization. Studies (Refs. 13 through 16) suggest this possibility, and a materno-fetal immunization rate of 5 to 11 percent has been observed (Refs. 14 and 16). Other studies have not confirmed these findings (Refs. 17, 18 and 38). Further studies are necessary to confirm the validity of the "grandmother theory." If proven, Rh-negative female children born of Rh-positive mothers might receive Rh_o immune globulin to prevent

such immunization and later instances of hemolytic disease in their children.

While not a factor strictly relevent to the Rh_o immune globulin prophylaxis failure rate, it is noteworthy that failure to administer Rh_o immune globulin still occurs. Estimates by the Centers for Disease Control indicate that only 80 percent of women who should receive immunoprophylaxis actually do so (Ref. 1). Clearly, further educational efforts are needed in the total program aimed at eliminating Rh hemolytic disease of the newborn.

Other situations in which Rh_o immune globulin may be useful include:

(1) *Rh_o immune globulin in abortion.* In cases of abortion, a risk of maternal immunization exists. The overall rate of immunization has been estimated as between 3 to 5.5 percent where the Rh type of the fetus is unknown (Refs. 19 and 20). The method of inducing abortion may influence this rate (Ref. 19); hypertonic saline abortion results in a three times greater frequency of transplacental hemorrhage than does curettage abortion. The risk of immunization also increase directly with gestational age. On the basis of these considerations, Rh immunoprophylaxis has gained general acceptance in cases of abortion. While controlled studies demonstrating the effectiveness of immunprophylaxis in abortion case have not been performed, the argument for use of Rh_o immune globulin in these cases is very compelling.

(2) *Rh_o immune globulin in transfusion.* The third major clinical circumstance where Rh_o immune globulin is used is when Rh_o(D) negative patients have accidentially received a transfusion of Rh_o (D) positive blood. Studies in volunteers indicate up to an 80 percent immunization rate in these circumstances and, administration of 20 micrograms of Rh_o immune globulin for each 1 mL of red blood cells affords total protection against immunization (Refs. 10 and 11). Other reports substantiate these observations (Refs. 21 through 24).

2. *Description of product.* Rh_o immune globulin is manufactured from human plasma containing anti-Rh_o(D) antibody. The plasma undergoes fractionation and the globulin fraction is obtained. The material is tested for specificity and potency in vitro in the latter case, by comparison with an FDA, Bureau of Biologics reference material. The final product may be diluted with serum globulin which does not contain anti-Rh_o(D). Stabilizing and antibacterial materials are added.

The Bureau of Biologics' requirements are that at least 200 mL each of plasmas (or sera) from 20 different donors be represented in the original plasma pool. While this is an apparently arbitrary number, it does permit some confidence that all specificities of the Rh_o(d) mosaic and antibodies of different affinity constants are represented in the final material. The Bureau of Biologics has limits for the presence of red cell antibodies other than anti-Rh_o(D) and these appear prudent and not overly restrictive.

The amount of anti-Rh_o(D) antibody is determined by an antiglobulin titer which must be equal to or greater than the Bureau of Biologics' reference material. This procedure is open to question. Manual titration using doubling dilutions is a highly subjective test. A one tube difference in such an assay system could reflect a 50-percent error in antibody concentration. Further, it has been demonstrated that assay values for Rh_o(D) antibody obtained by either manual or automated methods may be inaccurate as the regression line for the assay depends on both the antigen, i.e., the Rh-phenotype of the test cell, and the heterogeneity of the antibody population being tested. Finally, since the critical literature on efficacy of Rh_o immune globulin is expressed in doses described in micrograms of antibody, it would seem reasonable to demand consistency in standardization and expression of potency in the assay of manufactured lots.

Perhaps the best assay system for Rh antibody is the radioimmunoassay (Refs. 25 and 26). This test was the standard by which efficacy and dosage have been ascertained, and it has been demonstrably satisfactory. Radioimmunoassay procedures are difficult to carry out. A second quantitative technique that is acceptable is the Autoanalyzer™ titration method (Ref. 26). Since Rh_o immune globulin is prepared from plasma pools representing at least 20 different donors, the problem of antibody heterogeneity is reduced. When using this test, dilutions within one log are usually employed, and errors or inaccuracies are minimized.

3. *Effectiveness.* a. There are three clinical situations where administration of Rh_o immune globulin to prevent primary immunization to Rh antigen is currently used:

(1) *Postpartum in Rh-negative mothers who have delivered an Rh-positive child.* Administration within 72 hours after delivery affords 90-percent protection against primary immunization (Refs. 1 through 7).

(2) *After abortion in Rh-negative women.* Controlled studies are not available, but there is no reason to believe effectiveness is not equal to or greater than in term pregnancies.

(3) *After transfusion of Rh-positive blood to an Rh-negative individual.* Efficacy has been demonstrated in both controlled studies (Refs. 10 and 11) and clinical situations (Refs. 21 through 24).

b. There are a number of clinical situations where the use of Rh_o immune globulin has been suggested to prevent primary immunization:

(1) *After transfusion of blood components containing Rh-positive erythrocytes to Rh-negative recipients.* Transfusion of platelet or granulocyte concentrates is usually associated with infusion of substantial numbers of red cells. Primary immunization to the Rh antigen may be prevented with Rh_o immune globulin in those patients in whom it is desirable to do so, e.g., women in childbearing age. There is no evidence that cryoprecipitate from Rh-positive individuals can immunize Rh-negative recipients.

(2) *After abdominal trauma to an Rh-negative woman during pregnancy.* There is evidence that abdominal trauma, particularly amniocentesis, can be associated with feto-maternal bleeding (Refs. 28 through 32). It appears rational in these circumstances to administer intrapartum Rh_o immune globulin, although controlled studies are not available to support this use.

(3) *Intrapartum administration to all Rh-negative women.* Current evidence in a relatively small number of pregnancies suggests that primary immunization may be prevented with this approach, although not completely, (Ref. 8) as determined by postpartum Rh antibody detection (Refs. 2, 8 and 9). However, firm documentation of efficacy would require demonstration of no immunization with subsequent Rh-positive pregnancies. These data are not currently available. Such a program is directed at the 1.5 percent of at risk women who currently fail to be protected by postpartum prophylaxis. As the number of doses of Rh_o immune globulin would increase two- to three-fold with such a program, the impact on health care costs and on immunized plasmapheresis donors, the source of antibody-containing plasma, would be great if intrapartum prophylaxis were widely practiced. Thus, effectiveness should be established unequivocally before considering formal approval of this indication.

(4) *Administration to Rh-negative neonates born of Rh-positive mothers.* Current data do not support this use.

(5) *Administration to D^u women.* There are case reports of D^u women who developed "anti-D" with resulting

hemolytic disease of the newborn. This must be a rare event. Also, there is no evidence that administration of Rh_o immune globulin in these women would be protective; much of the administered antibody would be adsorbed by maternal cells (Ref. 34). Of greater concern is the clinical situation where the mother's Rh status is unknown prior to delivery. In these cases, a postpartum maternal typing as D^u might indicate the presence of a large feto-maternal hemorrhage of Rh-positive cells in an Rh-negative woman. In these cases, immune globulin should be given.

(6) *Administration later than 72-hours postpartum.* The 72-hour postpartum limit for Rh_o immune globulin resulted from an artifact in the experimental design during the original studies on this material (Ref. 7). This time limit appears valid for optimal protection but, if the antibody has not been administered within this period, it should still be administered later, as protection, albeit to a lesser degree, may still be achieved at little risk (Ref. 39).

c. *Miscellaneous issues affecting efficacy*—(1) *Estimating the size of feto-maternal hemorrhage.* The risk of feto-maternal immunization increases with the size of the feto-maternal hemorrhage (Ref. 2). The standard dose of 300 μg of Rh_o immune globulin protects against up to 15 mL of feto-maternal hemorrhage (Refs. 10 and 11). While hemorrhages greater than 15 mL occur in only 0.3 percent of pregnancies (Ref. 2), these women are at increased risk to become immunized and require more than the standard dose to be adequately protected. Provisions should be made in the labeling to recommend a screening or quantitative procedure for the detection of large (greater than 15 mL) feto-maternal hemorrhages. Appropriate upward adjustment of the dose of Rh_o immune globulin should also be recommended in these cases.

(2) *Low dose Rh_o immune globulin.* The current standard dose of Rh_o immune globuli 300 micrograms, is excessive in most cases (Refs. 5, 10, 11 and 42), although the safety of the product is not affected by the excess. During abortions or abdominal trauma, where the transplacental hemorrhage is generally far less than at term gestations, a lower does of Rh_o immune globulin may be equally efficacious. In Rh-negative male volunteers, it has been shown that 50 micrograms of Rh_o immune globulin completely protects against primary Rh immunization from a dose of 2.5 mL of red blood cells (Ref. 37).

4. *Safety.* Rh_o immune globulin has not been associated with severe toxicity. Localized reaction at the site of intramuscular injection is probably the most frequent side effect. A variety of complications have been reported in association with the administration of Rh immune globulin, but causal relationships are not always clear. Urticaria, temperature elevatio fever, myalgia, hyperbilirubinemia, splenomegaly, and rare sensitivities after repeated injections have been observed. Transmission of hepatitis has not been associated with this product.

Rh immune globulin administered during pregnancy to Rh-negative women carrying an Rh-positive fetus has not been associated with iatrogenic Rh hemolytic disease in the fetus (Refs. 2, 8, 9 and 33), although some infants show a positive direct antiglobulin test. Even when anti-Rh_o is administered to Rh-positive adults, there appears to be little hazard (Ref. 35).

5. *Benefit/risk.* Rh_o immune globulin is a highly effective product with few serious side effects.

6. *Recommendations.* a. The product should be placed in Category I for the currently approved indications.

b. In addition, Rh_o immune globulin should be approved for the following indications:

(1) After transfusion of any blood product containing Rh-positive red cells to an Rh-negative recipient in whom it is desirable to suppress primary immunization; and

(2) After significant abdominal trauma, including amniocentesis, to a pregnant Rh-negative woman.

c. Estimation of a large feto-maternal hemorrhage (greater than 15 mL) of Rh-positive cells should be recommended in all labeling as part of regular postpartum evaluation, and the dose of Rh_o immune globulin should be increased accordingly.

d. The 72-hour time limit for administering Rh_o immune globulin after exposure to Rh-positive red blood cells should be encouraged as preferable for prophylaxis. If more than 72 hours have passed, Rh_o immune globulin should still be given as soon as possible, up to 2 weeks after exposure to Rh-positive red blood cells, with the warning that efficacy may be reduced.

e. Rh_o immune globulin should not be given to:

(1) Rh-positive individuals,

(2) Rh-negative individuals known to be immunized to the Rh antigen, and

(3) in an Rh-negative woman where the fetus is known to be Rh-negative.

f. The requirement for Rh_o immune globulin crossmatch prior to administration should be eliminated.

g. Assay of all lots of Rh_o immune globulin must be performed by an approved method.

h. Encourage educational efforts to eliminate instances of non-administration of Rh_o immune globulin where it is indicated.

i. Further research is needed on (1) intrapartum prophylaxis, (2) immunization of Rh-negative newborns of Rh-positive mothers, (3) low-dose prophylaxis in abortion, (4) low-dose prophylaxis in abdominal trauma, and (5) the use of Rh_o immune globulin for intravenous use when large doses are required.

j. *Labeling recommendations.* (1) Add the new indications noted above.

(2) Add a recommendation for screening or quantitating the size of the feto-maternal bleed.

(3) Change the wording on the 72-hour time limit to permit administration for up to 2 weeks after exposure (Ref. 39).

(4) Eliminate the instructions for the crossmatch.

(5) Eliminate any statement directed specifically at administration to the infant.

(6) Eliminate the statement that Rh_o immune globulin should not be administered to "an Rh-negative woman who has received an Rh-positive transfusion within three months of delivery."

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17. Thrombin

Generic Statement

Thrombin is a proteolytic enzyme that initiates the conversion of fibrinogen to fibrin by splitting arginyl-glycyl bonds at the N-terminal end of the fibrinogen molecule (Ref. 1). As a result of this enzymatic action, two pairs of peptides, fibrinopeptides A and B, are released and the fibrinogen molecule becomes fibrin monomer. The molecules of fibrin monomer polymerize to form a fibrin clot. Thrombin has been reported to cause proteolytic activation of other enzymes and has esterolytic action on a number of synthetic amino acid esters as well (Refs. 2 through 5).

Thrombin activity can be determined by its ability to convert fibrinogen to fibrin, i.e., to form a visible clot or by its esterolytic activity (Ref. 3). It is derived from an inactive precursor, prothrombin, through the interaction of plasma procoagulants, calcium phospholipids (thromboplastin) (Refs. 6 through 8). Active thrombin is rapidly neutralized by several substances known collectively as antithrombins (Ref. 3). Even though considerable thrombin activity may be formed at a site of tissue injury, very little is present in the circulation.

Since thrombin acts directly on fibrinogen, it might appear that it would be an effective therapeutic agent for hemostatic disorders that are the result of defective conversion of prothrombin to thrombin. Thrombin has been found to be both unsafe and ineffective when given parenterally (Ref. 10). Under experimental conditions, dilute solutions of thrombin introduced into the circulation will produce disseminated intravascular coagulation (Ref. 10). When given intravascularly in more concentrated form, thrombin will cause thrombosis at the site of injection (Ref. 10).

Thrombin has been used topically as an aid to hemostasis (Ref. 10). There are several anecdotal reports indicating benefit from its use topically in accessible areas of bleeding from cancellous bone, dental extraction sites, skin-grafting sites, and sites of nasopharyngeal surgery. A few reports suggest that thrombin given orally along with a buffering agent is helpful in controlling bleeding of the upper gastrointestinal tract. No recent or comprehensive data were found concerning this clinical use.

Hemostasis results from interactions of platelets, the fibrin clotting system, and the vascular system (Refs. 11 and

12). At the site of vascular injury platelets clump together and adhere to the vessel wall to form a hemostatic plug. If the vascular defect is not too large, this platelet plug temporarily serves to prevent further bleeding. Later, the platelet plug is reinforced by a fibrin clot. Although the fibrin-clotting mechanism plays an inportant role in hemostasis and eventual repair of an injury, the platelet-vascular response is of primary importance for the prompt control of bleeding. Thrombin has been shown to play a role in platelet aggregation in vitro (Refs. 11 and 12), but there is no evidence that topically applied thrombin plays a direct role in the platelet reaction in vivo. It is well recognized that blood excaping from a denuded surface will promptly clot following the application of thrombin (Ref. 10).

However, the clot that is formed does not appear to prevent the loss of additional blood. Unless pressure is also applied, the clot formed in this way tends to be floated away by the escaping blood.

To provide a mechanism to apply pressure and to apply thrombin directly to the denuded surface, one technique employs a biodegradable sponge which is saturated with thrombin and applied to the surface of a wound (Ref. 23). Although this technique appears to aid in hemostasis, the Panel found no controlled clinical study to indicate whether hemostasis is achieved as a result of the use of the biodegradable sponge alone or as an effect of the thrombin or to both.

Thrombin is useful as a diagnostic reagent and as a reagent for research. The speed with which a visible clot forms is dependent on the concentration of fibrinogen and thrombin (Ref. 13). Under controlled conditions, the speed with which a visible clot forms after the addition of a standardized thrombin solution is a measure of the amount of fibrinogen present. This provides a rapid semiquantitative test for fibrinogen. It has been reported that individuals prone to have thromboemobolic disorders are deficient in antithrombin (Ref. 14). Thrombin is used as a reagent for the measurement of these antithrombins (Ref. 16). Although this test is promising, it is still experimental in nature.

Thrombin is prepared by separating prothrombin from plasma and then converting it to thrombin. Various methods have been used to obtain prothrombin; the principles of isoelectric precipitation, adsorption and elution, salt and alcohol fractionation, and chromatography have been utilized (Ref. 16). Prothrombin can be adsorbed from oxalated plasma by several poorly soluble salts such as barium sulfate, barium carbonate, aluminum hydroxide, and tricalcium phosphate. Prothrombin, along with other coagulation factors, can be eluted with sodium citrate from the adsorbents. The first high quality thrombin was prepared from bovine prothrombin by Seegers, et al. (Ref. 19), who used magnesium hydroxide as the adsorbing agent. Prothrombin was obtained by decomposing the magnesium hydroxide with carbon dioxide. Regardless of the method used, it is essential to separate prothrombin from the antithrombins that are present. Once this has been achieved, the prothrombin can be converted to thrombin by the addition of calcium and tissue thromboplastin. Unless further purified, these thrombin preparations contain at least trace amounts of other proteins. Commercial bovine thrombin has been used extensively as the starting material for the preparation of more purified thrombin (Refs. 18 through 21). In concentrated, lyophilized form, thrombin preparations are stable; once reconstituted to the liquid state, activity rapidly decreases (Ref. 15). If one is to rely on the unitage indicated on the label, freshly reconstituted solutions are essential.

Product Review

1. *Product.* Thrombin (Human) by Ortho Diagnostics, Inc. is a reagent for the qualitative determination of fibrinogen in human plasma. It is packaged in sealed ampules each containing 50 units of thrombin. It is to be reconstituted with 1.0 mL of saline. The recommended procedure is to add 0.2 mL of the reconstituted product to a 0.2 mL sample of plasma in a test tube. With normal plasma a visible clot will form in 15 seconds after the addition of thrombin, and the clot will adhere to the walls of the test tube. If the test plasma contains less than 100 mg/dL fibrinogen, the clot will be slower to form.

2. *Effectiveness.* Effectiveness is readily demonstrated by the routine laboratory control tests (Ref. 22).

3. *Safety.* The label carries a specific warning that this material is not for human administration and that it should be handled as if capable of transmitting hepatitis.

4. *Recommendations.* This product should be placed in Category I for in vitro diagnostic use only.

Product Review

1. *Description.* Thrombin Topical (Bovine) by Parke, Davis & Co. is supplied as a dried powder for solution containing 5,000 units of bovine thrombin, calcium chloride, sodium chloride, glycine, and benzethonium chloride. The potency is measured by determining its activity in clotting a standardized fibrinogen solution.

2. *Effectiveness.* Thrombin Topical (Bovine) is recommended for human use topically wherever oozing blood from capillaries and small venules is accessible to act an as aid to hemostasis. It may be used in conjunction with an absorbable gelatin sponge to aid hemostasis. Information submitted by the manufacturer (Ref. 23) tends to support this general indication. It should be pointed out that adequate, well-controlled clinical studies are not available in support of many of the specific indications mentioned in labeling or in the supporting literature. Much of the supporting data is anecdotal, but is based upon widely accepted clinical practices.

3. *Safety.* The label carries a warning that Thrombin Topical (Bovine) is not to be injected. When used as directed it appears to be safe.

4. *Recommendations.* a. The labeling should be modified to define more precisely the indications for clinical use. The manufacturer has suggested:

> Thrombin is indicated as an aid in hemostasis wherever oozing blood from capillaries and small venules is accessible. In various types of surgery, solutions of thrombin may be used in conjunction with absorbable gelatin sponge, USP, for hemostasis.

This statement is appropriate.

b. There are inadequate data to support the usefulness of thrombin administered orally for the treatment of bleeding peptic ulcer. The labeling should be modified to delete this as an indication for use.

c. With appropriate modifications of the labeling, it is recommended that this product be placed in Category I.

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18. Fibrinolysin and Desoxyribonuclease, Combined (Bovine), and Fibrinolysin and Desoxyribonuclease, Combined (Bovine) With Chloramphenicol

Product Statement

Product Reviews

1. *Introduction*—a. *Products.* Fibrinolysin and Desoxyribonuclease, Combined (Bovine), marketed by Parke, Davis and Company as Elase™ brand powder for solution and as Elase™ ointment.

Fibrinolysin and Desoxyribonuclease, Combined (Bovine) with Chloramphenicol, marketed by Parke, Davis and Company as Elase-Chloromycetin™ ointment.

For the purpose of this document, the brand name "Elase" will be used indicating the combination of Fibrinolysin and Desoxyribonuclease. Elase was issued a biological license in April 1960, and the Elase ointment with chloramphenicol was licensed in September 1963.

b. *Indications.* Elase lyophilized powder for solution is labeled by the manufacturer for topical use as a debriding agent in a variety of inflammatory and infected lesions. These include: (1) General surgical wounds; (2) ulcerative lesions—trophic, decubitus, stasis, arteriosclerotic; (3) second- and third-degree burns; (4) circumcision and episiotomy. Elase is used intravaginally in: (1) Cervicitis—benign, postpartum, and postconization and (2) vaginitis. Elase is also used as an irrigating agent in the following conditions: (1) Infected wounds—abscessed, fistulae, and sinus tracts; (2) othorhinolaryngologic wounds; (3) superficial hematomas (except when the hematoma is adjacent to or within adipose tissue).

Elase ointment is labeled for topical use as a debriding agent in a variety of inflammatory and infected lesions. These include: (1) General surgical wounds; (2) ulcerative lesions—trophic, decubitus, stasis, arteriosclerotic; (3) second and third degree burns; (4) circumcision and episiotomy. Elase is used intravaginally in: (1) Cervicitus—benign, postpartum, and postconization and (2) vaginitis.

Elase-Chloromycetin ointment is labeled for use in the treatment of infected lesions such as burns, ulcers, and wounds where the actions of both a debriding agent and a topical antibiotic are desired.

c. *Manufacturing.* Fibrinolysin is prepared from bovine plasma, isolated by ammonium sulfate fractionation, activated with chloroform, and precipitated by adjusting the pH to the isoelectric point. The desoxyribonuclease is prepared from bovine pancreas by a chromatographic isolation technique. Potency standardization for each ingredient is done on the raw material and on the final product. Proteolytic activity is measured by a fibrinolytic assay measuring the time that it takes for a clot of bovine fibrinogen to be lysed. Desoxyribonuclease is measured by a viscometric assey in which the product is allowed to act upon desoxribonucleic acid (DNA) substrate.

d. *Pharmacology.* The pharmacologic rationale for the combination of these two enzymes is based on the observation that purulent exudates consist largely of fibrinous material and nucleoprotein. Desoxyribonuclease attacks the DNA and also chromatin; therefore, theory would predict that it should lyse exudate that is rich in cell nuclei. Fibrinolysin is an endo-peptidase which ruptures peptide bonds in proteins in which lysine is the amino acid and should thus lyse any clotted protein of plasma origin. Chloramphenicol, a broad-spectrum antibiotic, is included in the Elase-Chloromycetin ointment for use in the treatment of infected lesions where the action of a topical antibiotic "may be helpful."

2. *Evidence of effectiveness.* Data supporting the effectiveness of Elase covers a wide variety of conditions and types of studies. The primary rationale for use is based on the theory that lesions needing debridement might heal better with enzyme therapy. The clinical trials vary from double-blind, controlled studies to anecdotal reports on a few patients.

The most convincing data supporting the effectiveness of Elase comes from the study of Friedman et al. (Ref. 1) on the treatment of post-partum cervicitis. When Elase was used as a solution at each of two dose levels, and compared with a placebo, there was no difference in results. However, when Elase was incorporated in an ointment base and administered more frequently, there was a significant difference between the placebo ointment and Elase ointment in favor of the Elase. This was reflected in a decreased need for post-partum cervical cauterization as well as improvement in other parameters which were evaluated. In these studies there was no additional benefit evident from including chloramphenicol in the ointment.

The other controlled and blinded trial was by Glick et al. (Ref. 2) on postconization cervices. The

investigators reported little difference between Elase and placebo but found the inclusion of chloramphenicol to be beneficial. Since no group was treated with chloramphenicol alone, it was not known whether Elase is of any benefit for this indication.

Kaufman et al. (Ref. 3) in an unpublished study investigated the effect of Elase, Elase plus chloramphenicol, and a placebo upon "postpartum" bacterial cervicitis. There was no statistical difference between the three groups according to a Parke-Davis memorandum (Ref. 3), although it is not clear how the therapeutic results were evaluated. Chloramphenicol did not seem to have any effect on the type of flora seen after treatment.

The other uncontrolled gynecologic studies are of little help in evaluating drug effectiveness. It was noted that in one unpublished study of the chloramphenicol-containing ointment, there seemed to be an increased incidence of vaginal moniliasis (Ref. 4). In this report, 10 percent of the patients were dropped from the study because their white blood count (WBC) had dropped below normal after 2 days of treatment. No mention of this phenomena was made in other studies, although this may be because serial WBC's were not done.

The evaluation of Elase treatment on other surgical lesions has not been reported in well-controlled clinical studies. Several investigators have reported the effect of these agents on chronic lesions unresponsive to standard therapy and have concluded that there is a beneficial effect. The report of Coon et al. (Ref. 5) is an example of this type of study. Patients with chronic ulcers or abscesses were treated with Elase solution or ointment. Ulcers associated with arteriosclerosis showed little response to treatment and none of these healed, whereas trophic and decubitus ulcers were reported to be treated successfully. Four burn patients also were reported as responding well to Elase ointment. Similarly, Connell and Rousselot (Ref. 6) discussed treatment of 125 patients of whom 90 percent were judged to respond to this treatment in 24 to 72 hours. The authors of other studies of this nature all conclude that enzymatic debridement is a useful treatment.

The only available data on the use of Elase in circumcision and episiotomy were found in one table in the paper of Margulis et al. (Ref. 7). In this report, all of the 30 standard treated circumcision patients are classified as healing more slowly than patients treated with Elase ointment for 4 to 6 days. This same report lists eight patients with "inflamed" episiotomy wounds which "healed" quickly when enzyme ointment was applied.

The recommendation for use in otolaryngology is supported by several letters from Dr. Sylvester Daly (Ref. 8) which discuss the use of Elase in a few patients. Analysis of these data is not possible.

One article reports on the treatment of 107 ophthalmic lesions with Elase (Ref. 9). The author concludes "in no instance was healing impaired——. In dendritic keratitis and severe trauma healing was speeded up and morbidity diminished."

During the original licensing of this family of products, the then current regulatory agency, the Division of Biologics Standards of the National Institutes of Health felt the controlled trial indicated fibrinolysin with desoxyribonuclease to be effective, although no clinical data supported the advantage of the combination of fibrinolysin and desoxyribonuclease as compared with the individual components. As shown by the correspondence between the regulatory agency and Parke-Davis (Ref. 10), there was concern about the usefulness of incorporating chloramphenicol into this same ointment. None of the studies then available supported this view. The later report of Glick et al. (Ref. 2) seems to support this contention after conization of the cervix. However, there is no evidence to support the effectiveness of Elase in this investigation. Chloramphenicol ointment alone might have given the same results without the reported side effects of increased bleeding. The Friedman study (Ref. 1) shows no significant difference in favor of chloramphenicol nor does the study at Baylor (Ref. 3).

3. *Evidence of safety.* A review of the studies referenced above indicates that the fibrinolysin-desoxyribonuclease combination exhibits a high degree of safety. During the original licensing there was concern about sensitization of patients to the enzyme proteins of bovine origin. From the data submitted this does not seem to be a problem. Bleeding after conization is an expected sequela of this type of treatment. The report of decreased WBC's in several patients receiving the ointment containing chloramphenicol should be further investigated. There is a report of at least one case of a blood dyscrasia associated with an ophthalmic chloramphenicol ointment, and there is probably significant absorption of chloramphenicol through mucosal and damaged wound surfaces.

4. *Benefit/risk evaluation.* Published studies about Elase and experience with the product indicate that it is probably effective as a general debriding agent. The data on circumcision, episiotomy, and certain other specific indications are still not convincing. The inclusion of chloramphenicol in the ointment remains of concern. None of the studies offer significant evidence of benefits from the combined enzyme-antibiotic compared to the separate ingredients.

The following conclusions were reached:

a. For the Elase ointment with chloramphenicol, there was relatively little bacteriologic data indicating that there is a need for the antibiotic or demonstrating its effectiveness. The only bacteriological studies reported were essentially surface cultures. Newer methods, such as punch biopsies of eschars, were not done to find out whether the chloramphenicol does anything more than cause the surface contamination to be somewhat suppressed.

b. Systemic absorption of the chloramphenicol component and its potential adverse reactions were a cause of concern. Some of the recommended indications would allow a large amount of the ointment to be placed in a body cavity where significant amounts of chloramphenicol could be absorbed, particularly if the application is repeated frequently over a period of several days. It was noted that no hematologic studies have been done on absorption or systemic effects of Elase with chloramphenicol. White blood counts concurrent with treatment may be indicative of a reaction to chloramphenicol even though the development of aplastic anemia may not be detectable until some time after cessation of therapy.

c. The safety of the two enzyme ingredients either in the ointment or in the solution were not questioned. There was no evidence of sensitization resulting from the enzymes.

d. Regarding effectiveness, there are only one or two well-controlled trials. Most of the other reports were more of a testimonial nature. Also, most of the studies were conducted some years ago. With the variety of conditions treated with these products, the progress of wound healing is difficult to assess unless there are adequate controls. Factors such as degree of surgical debridement, frequency of dressing changes, proper cleansing, and other nursing care practices affect the outcome and must be well controlled to provide an objective evaluation. Many of the indications in the current labeling may be treated by alternate methods, some of which have been developed

since the original studies were done with these products.

e. Therefore, the benefit-to-risk ratio may not be as favorable now as it was at that earlier time. Although there has been extensive use of the products and sales have been increasing over the years, this is not significant evidence of effectiveness. However, it may have some relevance in the judgement of safety.

5. *Recommendations*—a. At the request of the Panel on Review of Blood and Blood Derivatives the Bureau of Biologics, the Surgical Drugs Advisory Committee of the Bureau of Drugs first reviewed the Elase products at meetings held December 15–16, 1977 and March 17, 1978. Although it was recommended by the Surgical Drugs Advisory Committee that Elase powder for solution and Elase ointment be placed in Category I (with the provision that the labeling be modified to remove those specific indications for which there are insufficient data), the Panel on Review of Blood and Blood Derivatives does not concur with this recommendation, but rather recommends that these products be placed in Category IIIA. This recommendation is based upon the combination policy stated in 21 CFR 601.25(d)(4) and is consistent with recommendations made for other combination products. Evidence should be developed by appropriate in vivo or in vitro tests to demonstrate that each component makes a contribution to the claimed effects. A period of 3 years is recommended for the completion of these studies.

b. Indications which are recommended to be deleted if the products remain on the market pending the completion of studies inlcude:

(1) Trophic, decubitus, stasis, and arteriosclerotic ulcers.

(2) Second- and third-degree burns.

(3) Circumcision and episiotomy.

(4) Vaginitis.

(5) Specific use as an irrigating agent in: Abscesses, fistulae, and sinus tracts; otorhinolaryngologic wounds; and superficial hematomas.

c. Elase ointment with chloramphenicol (Elase-Chloromycetin™] should be placed in Category IIIA because there are insufficient data demonstrating effectiveness of this combination product. Additional studies should be completed to demonstrate the effectiveness of the combination product. It is recommended that a period of 3 years be permitted for completion of the studies. The primary study should be a double-blind clinical trial to compare the safety and effectiveness of Elase-Chloromycetin ointment to Elase ointment and to a placebo in a management of a variety of infected skin lesions. The study should be designed to show that each individual ingredient contributes to the claimed effects of the combination. During the period the studies are being conducted, the labeling should be modified to remove the indication for use in burns.

During the deliberations of the Surgical Drugs Advisory Committee, Parke-Davis and Company provided details of a suggested protocol and suggested modified labeling (Ref. 11). It is recommended that the Bureau of Biologics review these submissions. In addition to the application of the protocol suggested by the manufacturer, it is recommended that more objective means to measure wound healing be employed.

This should include photographs taken under controlled conditions and the use of tracings which may be transferred directly to records. Also, wound culture methods should be designed to detect the presence of anaerobic organisms. Swab cultures alone are considered to be inadequate. There is also a need for thorough follow-through of all patients accepted into the study and for careful design for the evaluation of results.

d. Regarding the potential for adverse reactions from chloramphenicol absorption from the wound into the systemic circulation, a separate, smaller study is recommended, selecting patients with more extensive wounds which require larger amounts of the Elase-Chloramphenicol ointment.

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19. Blood Grouping Diagnostics

Generic Statement

1. *Introduction.* The clinical and laboratory investigations that followed discovery of the ABO and Rh blood groups have shown clearly that blood almost always can be infused without acute hemolytic reactions if certain laboratory tests are done before transfusion. These are (1) ABO grouping, (2) Rh typing, (3) testing of donor and recipient for selected blood group antibodies and (4) testing, by means of a crossmatch procedure, for potential incompatibility between donor and recipient. Licensed reagents for these tests include (1) blood grouping serums, (2) reagent red blood cells (human) and anti-human serum. These are used with samples of serum or red cells to do the testing required for some of the processing of donor blood, typing of recipient blood, detecting antibodies in donor serum, and finally, crossmatching between the intended donor and the recipient. All these tests are based on the principles of immunologic reactions involving antigens and antibodies.

The blood group antigens are complex biochemical structures that make up part of the red cell membrane. The corresponding antibodies are similar to other antibodies in that they are formed as a result of previous exposure to a specific antigen and, like other antibodies, they react with these antigens under appropriate conditions. The resulting antigen-antibody complex provides an indicator for the blood typing, antibody detection, or crossmatching tests.

In the laboratory a reaction between red cell antigens and the appropriate antibodies results in one or more of the following events (Ref. 1): (1) Hemolysis (destruction) of the cells, (2) agglutination (clumping) of the cells and/or (3) coating of red blood cells with antibody without demonstrable hemolysis or agglutination.

When hemolysis or agglutination occurs in a crossmatch test for example, positive tests are easily noted and are clear indiators that the recipient's antibody involved may be clinically important. Not all dangerous antibodies, however, react with red cells to produce such obvious evidence of their hazardous potential. In many cases, red cells become coated with antibody (i.e., are sensitized) without demonstrable hemolysis or agglutination. When this

sensitization occurs in vivo the antibody coated cells are frequently recognized by the body as incompatible and are removed rapidly (Ref. 2). Such antibodies can usually be detected by the antiglobulin or Coombs test. This test depends upon the fact that the reaction between red blood cells and these antibodies leaves the cells coated with antibody protein, or with various complement components, or with both. These red cell-bound proteins are detected most easily and reliably by the antiglobulin test in which the cells are reacted with Anti-Human Globulin Serum as discussed later in this section.

2. *The complement system.* Immunologic reactions between red blood cell antigens and their corresponding antibodies involve, at times, a family of plasma proteins known as the complement system (Ref. 3). In the blood stream the complement-antibody-red cell interaction often causes antibody coated red blood cells to be removed from the circulation. In the test tube a reaction between antibody and red blood cells can also involve proteins of the complement system when they are present in active form.

In vitro, complement-antibody-red cell interactions lead to two possible consequences (Ref. 4).

a. If the antibodies are of the complement-fixing variety, red cell hemolysis may occur. Such antibodies are usually IgM, but some antibodies, especially anti-A, in the IgG class also cause hemolysis in this way.

b. Certain antibodies, particularly IgM cold agglutinins, may react with red cells and, as part of this reaction, fix some complement proteins to the red cell membranes. Under some conditions, the bond between the two blood cells and the antibody is subsequently broken, leaving the cells free of antibody but coated with complement components. Hence, a test under these circumstances to detect the original antibody-red cell interaction will be negative unless it is also capable of detecting the presence of the appropriate complement components on the red cells.

The Complement Reaction Sequence

The complement system consists of at least 11 different proteins that interact in a sequential fashion. Complement components are abbreviated by the capital letter C and an appropriate numeral from 1–9, e.g., C1, C2, C3, etc. The addition of a lower case letter, e.g., C3a, C3b, indicates fragments of the components and a bar over the term indicates the activated form, e.g., $\overline{C1}$, $\overline{C2}$, $\overline{C3}$, etc. These components interact in a numerical sequential fashion except for C4 which reacts with $\overline{C1}$ so that the reaction sequence becomes C1, C4, C2, C3, C5 through C9.

This reaction sequence, the so-called classical pathway, can be initiated by diverse biochemical or immunological reactions, but only those related to red cells and blood group antibodies will be considered here. A second reaction sequence, called the alternative pathway, has been described but will not be included in this discussion since current evidence indicates that it is not related to immunological reactions that are important in blood transfusion practice. The reactions can occur in vivo or in vitro and do not always involve the complete sequence of complement proteins. Three phases of the reactions, recognition, attack, and lysis, have been defined. In addition, reaction control mechanisms are present and include either decay of selected components or inhibition of further reactions by specific inhibitors.

a. *Recognition.* The binding of one IgM or two adjacent IgG blood group antibody molecules to a red cell membrane is sufficient to initiate the complement reaction sequence. Calcium links C1q, C1r, and C1s into a macromolecular complex. The C1q fragment of this complex reacts with cell bound antibody and is activated to form $\overline{C1}$ which, in turn, acts on C4.

b. *Activation.* The action of C1, particularly the $\overline{C1s}$ fragment, converts C4 to $\overline{C4b}$ by splitting off a fragment known as C4a. $\overline{C4b}$, in conjunction with the previously established C1qrs-antibody-red cell complex binds to an adjacent location on the red cell membrane. Here the $\overline{C4b}$ combines with $\overline{C2a}$ which had been previously generated by the action of $\overline{C4b}$ on circulating C2. $\overline{C4b}$, $\overline{C2a}$ and magnesium establish the $\overline{C4b,2a}$ activation enzyme which next acts on C3.

c. *Lysis.* C3, under the influence of $\overline{C4b,2a}$ is activated to C3b by enzymatic removal of the C3a peptide. Some C3b molecules join the $\overline{C4b,2a}$ complex to establish a new complex C4b,2a,3b, which reacts with C5, activating it to the C5b form and releasing C5a. Other C3b molecules do not participate in the formation of $\overline{C4b,2a,3b}$ complexes but instead are acted upon by C3 inactivator and converted to C3d (Ref. 5). C3d is a cell-bound component. Although it is apparently biologically inert, its presence can be used as a reliable indicator of previous antibody-red-cell interactions (Ref. 6). When the $\overline{C4b,2a,3b}$ complexes bind to cells there may be a progression of the activation sequence through the C9 state ending in cell lysis. In some cases, the sequence stops at C3, so that red cells are coated with C proteins but not lysed.

3. *Blood grouping serum*—a. *Introduction.* In 1900 Karl Landsteiner observed that the serum of normal humans frequently agglutinated the red blood cells of other individuals. A year later he reported that the reactions showed a "peculiar regularity" which separated human blood into three phenotypes. It is now known that these agglutination reactions were the visible indication of interactions between antibodies in the serum of one individual and the homologous red blood cell antigens of another. The phenotypes he described were A, B and O, although these letter designations were not universally accepted until many years later.

Immediately, Landsteiner and others suspected that human red cells might possess more than the two unique antigens, A and B, already observed and began a search for new blood groups. Looking for additional blood group antibodies, these early workers tested many serums from normal persons, but none were discovered by the methods available at that time. They turned, then, to the injection of human red cells into laboratory animals in an effort to stimulate production of antibodies that would identify new blood groups. These experiments were successful, and the P and MN blood groups were detected and reported in 1926. Shortly thereafter, antibodies specific for P and M were also found in human serum, but it was not until 1940 that the important Rh blood group system was recognized. The Rh antigens, like nearly all of the 400 or more blood group antigens now known (Ref. 7), were detected because the corresponding antibodies were found in the serum of a patient. The Rh antibodies are almost always stimulated by transfusion or by pregnancy. In the latter case, small numbers of fetal red blood cells cross the placenta, enter the maternal circulation, and induce production of one or more blood group antibodies.

b. *Source.* Blood Grouping Serum is serum containing a blood group antibody which, when used as directed by the manufacturer, will detect the presence or absence of one or more specific blood group antigens. Most Blood Grouping Serum is prepared from human rather than animal sources, although some reagents prepared in rabbits and goats are currently licensed. In some cases, plasma is harvested from persons who have been immunized by pregnancy or transfusion and have produced antibodies that can be processed into a suitable reagent.

Nearly all of the less widely used and less important blood grouping serums (e.g., Anti-Kp^a, Anti-Kp^b, Anti-S, Anti-s, Anti-Di^a, etc.) are prepared from such starting material. Other blood grouping serums (e.g., Anti-A, Anti-B and Anti-D) are needed in such large quantities or must be of such potency (see below) that additional stimulation of selected donors is required. In such cases, donors are stimulated by injection with blood group substances or appropriate red cells and, if suitable antibodies result, these donors are then plasmapheresed on a regular schedule to obtain Source Plasma (Human) for further processing into Blood Grouping Serum. As mentioned earlier, only a few blood-grouping serums, such as anti-M, anti-N, anti-P$_1$, anti-Le^a and anti-Le^b, are prepared by immunization of laboratory animals.

c. *Indications for use*—(1) *Pretransfusion testing of recipients.* Before transfusion, it is essential to test the red blood cells of the recipients with anti-A, anti-B, and anti-D to determine their ABO groups and Rh(D) types. Many other Rh blood antigens are known, and Blood Grouping Serums for some of these are easily available (anti-C, anti-E, anti-C^w, anti-c, anti-e, etc.), but only tests for the D antigen are required for routine purposes. The reasoning for this decision has been developed in the section on Rh typing under Whole Blood (Human).

(2) *Testing of donor blood.* All donor blood must be characterized as to ABO group and Rh(D) type. To accomplish this, the donor red blood cells must be tested with anti-A, anti-B and anti-D. Routine tests for other blood groups are neither required nor advised.

(3) *Pretransfusion testing of donor red cells when the recipient has been previously immunized.* The serum of certain recipients who have been previously transfused or pregnant will contain one or more blood group antibodies. Such persons must be transfused with blood lacking the corresponding antigen if a transfusion reaction is to be avoided. The compatibility test or crossmatch is an important procedure because it can usually detect evidence of potentially dangerous antibodies in the recipient's serum. When the specificity of the antibodies is known and an appropriate blood-grouping serum of the same specificity is available, it is useful to test red cells from the intended donor to be certain that they lack the antigen in question. Such tests, of course, depend upon previous identification of the recipient's serum antibody (see section on Reagent Red Blood Cells).

(4) *Testing of red blood cells for genetic, medicolegal, or research purposes.* Because red blood cell antigens are inherited characteristics and because they do not, for all practical purposes, change throughout life, their presence or absence can serve as useful genetic markers. Red cell antigens, for example, have been used to exclude paternity, identify blood stains, determine twin zygosity, and establish family relationships. Such studies and others rely on blood grouping serums.

d. *Safety and effectiveness.* The effectiveness of any blood-grouping serum is evaluated by its ability, when used as directed by the manufacturer, to type correctly the blood cells being tested. A positive test result is indicated by agglutination (i.e. clumping) of the red cells. An ideal serum is one that is both highly specific and sensitive. Specificity refers to the ability of the serum to react with a given blood group antigen but with no other. Since Blood Grouping Serum is nearly always derived from the plasma of individuals who possess antibodies against more than one blood group antigen, a manufacturing process is required to remove or neutralize the unwanted antibodies. Appropriate tests on the final product are essential to show that unwanted antibodies have been removed and additional tests are required to show that the original specificity is retained at the end of the dating period. In some very rare cases the desirable antibodies do not maintain adequate reactivity throughout the storage period, while activity of antibodies that originally had been adsorbed or inactivated return.

Sensitivity, the capability of a blood grouping reagent to detect the weaker reacting red cell antigens of the claimed specificity, is an equally important characteristic of licensed Blood Grouping Serums.

While many individuals develop blood-group antibodies as a consequence of transfusion or pregnancy, only a few produce serums with sufficient potency to be useful as blood grouping reagents. Unlike chemical reagents that can be measured in absolute terms, standards of potency for blood-grouping serum can be established only by comparing the test serum with a reference serum by titration, a test that applies subjective criteria concerning the strength of the agglutination produced by increasing dilutions of serum. In the usual titration a series of tests is done; each succeeding tube in the series contains half the amount of serum present in the previous tube. The end point is recorded as the greatest serum dilution (e.g., 1:2, 1:4, 1:8, 1:16, 1:32, etc.) in which test cells are agglutinated. The reaction strengths are scored by one of several arbitrary grading systems. Federal regulations (Ref. 8) mandate that actions be graded as follows:

4+—cell button remains in one clump;
3+—cell button dislodges into several clumps;
2+—cell button dislodges into many small clumps of equal size;
1+—cell button dislodges into finely granular, but definite small clumps; and,
D—cell button dislodges into fine granules but not definite small clumps. Results shall be recorded as doubtful.

Requirements for both sensitivity and specificity of Blood Grouping Serums are arbitrary but have resulted in reagents that are reliable and effective. For some reagents (e.g., anti-A, anti-B, anti-D, anti-A,B, anti-CD and others) reference serums are available from the Bureau of Biologics. Manufacturers must demonstrate that their reagents are at least as potent as these reference standard serums. For other blood grouping serums reference standards are not supplied but regulations define minimum potency standards that such serums must meet. These standards are expressed in terms of the titration value that a serum must reach to be acceptable. Reagents that are generally in good supply such as anti-K, anti-k, anti-Fy^a, and anti-C^w must show at least a 1+ reaction with a 1:8 dilution of the antiserum. Others, such as anti-S, anti-s, anti-P$_1$, anti-M, anti-I, anti-e (saline), anti-c (saline) and anti-A$_1$, need react only at a 1:4 dilution; antibodies in even shorter supply are acceptable if a 2+ reaction is seen with undiluted serum. Clearly these regulations suggest that weaker reactions are acceptably safe, at least for reagents that are used infrequently and in special circumstances. There are no controlled studies to show that blood grouping serums which meet current regulations are effective, nor are any now needed. The vast clinical experience gained from the millions of accurate blood typing tests done each year indicates a high level of effectiveness for the reagents, as currently prepared.

Whether reagents meeting a different standard, for example, more diluted, would be equally acceptable is unknown. The question is important since the starting material for production of Blood Grouping serum almost always requires hyperimmunization of donors, a procedure not without risk as discussed

elsewhere in this report (See Donor Immunization and Hyperimmunization). If satisfactory reagents can be prepared using smaller amounts of starting plasma, the benefit will be obvious. Unfortunately, the required data relating to effectiveness are not available.

The Panel recommends that studies be designed and instituted to determine the lowest titer of antibody that will produce an effective agent. Such studies should especially address the production of anti-A, anti-B, anti-A,B, anti-D and other Rh antiserums.

Current Federal regulations (21 CFR 660.26(c) (1), (2) and (3)) define specificity tests to ensure that Blood Grouping Serums are free from unwanted or contaminating antibodies. The Panel believes that requirements to test candidate serums against red blood cells containing some antigens, e.g., Co^b, Do^a, Do^b, Yt^b, and M^g, will be difficut to meet since antiserums to prove that test cells possess these antigens are not easily available. Thus, it is recommended that these requirements be reviewed and modified.

e. *Methodological considerations.* (1) There are several commonly used methods for testing blood types. Serums prepared and standardized to produce acceptable results by one method may, when used by another, give weak or even incorrect answers.

(a) *Slide or rapid tube tests.* Blood grouping serums for these tests must react appropriately when tests are done on slides or in tubes. When slides are used, a red cell suspension of approximately 50 percent in autologous or compatible serum or plasma usually is required, although some manufacturers specify cell concentrations of 5 to 15 percent. For some serums (e.g., anti-C, anti-D and others) results must be interpreted by observing the test over a heated viewbox. Such serum usually contains a potentiating agent such as bovine serum albumin. Slide tests are generally less sensitive than tube tests and are not frequently done. There is no evidence, however, that properly performed slide tests are inaccurate, and there is no reason to suggest that reagents for slide tests are unsafe.

When blood from some *patients* is tested with anti-D by slide tests, false-positive results may be seen. Similar considerations apply to modified tube tests. These false results are caused by clumping of the patient's cells by his or her own serum or by some component of the reagent other than the antibody itself. It is essential to provide a control for these false positive results by simultaneously testing the individual's blood alone and with the diluent medium of the blood grouping serum. Recently it has become clear that bovine serum albumin solutions are not ideal for this control purpose (Ref. 9) probably because some marketed reagents contain agglutination-potentiating substances other than albumin. The appropriate control material is the serum diluent medium itself added to serum lacking the specific antibody. Such control materials add a small but definite measure of safety to blood-grouping tests. Manufacturers should be required to make appropriate control materials available for use with their anti-D serum.

When testing donors it is also possible to get false-positive results by the same mechanism. Because donors are almost always healthy, such problems are infrequent. Furthermore, they do not compromise patient safety since the worst that can happen is the classification of an Rh negative donor as Rh positive. As such his/her blood will be given to an Rh positive recipient. But, as is well known, the use of Rh negative blood in Rh positive recipients is without added risk. Hence the Panel does not believe that the added cost of an Rh control is justified in the testing of donor blood.

"Modified tube" tests are done using red cells suspended in serum or plasma. While the relative amounts of cells and serum in the mixture are not critical, acceptable ranges should be defined in the instructions to the user. False-positive reactions, as described above, may be seen and a control reagent lacking the antibody should be available.

(b) *Tube tests.* Red blood cells, suspended in saline or in compatible or autologous plasma, may be tested by any of several methods including:

(i) Incubation at 37°C and centrifugation,

(ii) Incubation at 4°C and centrifugation,

(iii) Incubation at room temperature without centrifugation,

(iv) Immediate centrifugation and reading without incubation,

(v) Incubation at 37°C, washing, and testing with Anti-Human Serum (see below), and

(vi) Some combination of above.

Tube tests are commonly used for most blood bank work. Methods that can be completed without long incubation times or additional procedures such as the use of Anti-Human Serum are preferred for routine tests such as those for A, B, or D. Other methods, including those that use Anti-Human Serum, are used when simpler procedures are not sufficiently sensitive.

(c) *Tests with automated equipment.* Two automated blood-typing machines are currently available. These machines require reagents that are prepared specifically for this purpose since reagents that meet standards for tube or slide tests may not yield satisfactory results when used by automated methods. Licensed reagents for such equipment must, after a two-fold dilution, be potent enough to detect the antigen on heterozygous red cells.

f. *Labeling and packaging*—(1) *Packaging.* Blood grouping serums, excluding those intended for use in automated equipment, are packaged in dropper vials containing between 1 and 10 mL of reagent. Suggestions that packaging should be permitted in amounts greater than 10 mL have been rejected (Ref. 10) because of the potential for error that accompanies the transfer of reagents from larger to smaller containers. The Panel recommends that bulk packaging for some reagents, e.g., anti-A or anti-B, is reasonable for some large users and should be allowed.

The dropper bulb must be black or color matched with labels of reagent bottles that allow for color coding. Suggestions that white dropper bulbs be allowed have been rejected (Ref. 10) because of the desire to restrict the use of white dropper bulbs to Anti-Human Serum. The Panel agrees that these cautions are justified and supports the current regulations as written.

(2) *Labeling.* Labeling for blood grouping serums includes the vial label, the package label if a package is provided, and the package insert. Since some vials contain only 1 or 2 mL of serum, the amount of space on the label itself is restricted and regulations require that only a limited amount of information appear on the label. Additional information may appear either on the container label or the package label. The remainder of the information, including directions for use, may be provided in the package insert. This procedure is satisfactory, and the Panel sees no need to suggest major revisions.

g. *Other recommendations.* (1) The Panel concludes that the reviewed Blood Grouping Serum products should be placed in Category I.

(2) Current labeling arrangements with some information on the vial label, some on the package, and some included in the package insert are appropriate. The Panel recommends that the vial label, because of its small size, be reserved for critical information concerning product identification and

safe storage procedures, the lot number, and the manufacturer's name.

4. *Reagent Red Blood Cells*—a. *Introduction.* Reagent Red Blood Cells are prepared from selected human red blood cells and are intended to detect or identify human blood group antibodies. The blood from which these cell preparations are made is collected, diluted with an anticoagulant preservative solution, packaged, and distributed. The blood types of the red cells, the packaging, and the circular of instructions determine, to a large extent, the ultimate use of the cells. For example, A and B cells are universally used in the reverse-typing or serum-grouping test to confirm the ABO type determined by cell-typing methods, while O cells are used for either antibody detection or antibody identification outside the ABO blood group system.

b. *Source.* Reagent Red Blood Cells from adults are collected by producers that meet applicable regulations applied to donors of human blood for transfusion. These regulations ensure the safety of both donor and product. Since the same procedures cannot be applied to the collection of blood from newborn infants, manufacturers of cord red cells, which are used for tests of specificity in the I_i blood group system, must collect them in institutions that have large obstetrical services. One donor (i.e., one umbilical cord) can provide only a limited number of red blood cells; thus, products prepared from cord red cells, unlike those from adults, consist of pools from many infants.

c. *Indications for use.* (1) *As reagents to assist in determination of the ABO group of patients or blood donors.* Almost all adults normally have, in their plasma, antibodies against the A and B antigens that are absent from their own red blood cells. For example:

Blood group	Red blood cell antigens	Serum antibody
A	A	Anti-B.
B	B	Anti-A.
O	Neither A nor B	Anti-A, anti-B.
AB	A and B	Neither anti-A nor anti-B.

It is appropriate to confirm the ABO typing of patients or of blood donors by testing their serums for the expected antibody. Reagent Red Blood Cells of type A and type B are used for this purpose. The Type A cells must be of the A_1 subtype since the anti-A antibodies in the plasma of group B and group O persons always possess this specificity. Rarely, persons of type A_2B and even more rarely persons of type A_2, have antibodies that react specifically with cells of the A_1 subtype. In such cases, additional testing with A_2 cells may be helpful but is not essential.

(2) *As reagents for the detection of unexpected blood group antibodies.* It is common practice to test the serums of patients and blood donors for clinically important blood group antibodies. This testing, also known as "antibody screening," is done by mixing the serum to be tested with Reagent Red Blood Cells intended for this purpose and subjecting the mixture to one or more tests for an antigen-antibody reaction. These "screening cells" must be of type O since antibodies of other than A or B specificity are being sought by this test.

Issitt and Issitt (Ref. 7) estimate that there are at least 400 different blood group antigens. The red blood cells of any one blood donor can be expected to posses only a proportion of these, although antibodies to most may be dangerous. Blood group antibody screening tests of patients should be able to detect as many as possible of the antibodies that can occur and, of course, must detect those that are clinically most important.

Federal regulations require that Reagent Red Blood Cells used for detection of unexpected blood group antibodies must contain at least the following antigens: D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P_1, M, N, S, and s. Because it is impractical to find one donor whose cells have all these antigens, tests for antibody detection must be done using cells from two or more donors. These cells may be pooled or used individually, but in either case must together provide all the required antigens. If the test cells are pooled, the final pool must be prepared from equal volumes of cells from no more than two donors. If reagent cells are provided individually, each lot, but not each cell sample in the lot, must have cells positive for each of the listed antigens.

The Panel believes that the goal of having each required antigen present on half of the cells in the final pool is appropriate. Limiting the pool of cells to two donors, as currently is done, is the best way to do this since it reduces the possibility of weak and misleading mixed field reactions.

Because there is a general opinion, unsubstantiated by data, that weakly reacting antibodies are more easily detected using individual rather than pooled cells, the use of pooled cells for testing patient serums has become infrequent. The disadvantage of using individual cells is that each patient's serum must be tested at least twice with the resultant increase in cost and labor. In the case of tests of donors, there is good evidence (Ref. 11) that problems caused by antibodies in donor serum are so infrequent as to warrant a different approach, and antibody testing with pooled cells is acceptable in this situation. This is in part because donor blood, when transfused, results in an immediate dilution of the donor antibodies by the recipient's plasma; this leads to at least a give-fold diminution in their concentration. In addition, the transfused antibodies encounter a large excess of antigen-bearing red cells. As a result, rarely, if ever, is the blood cell antigen to antibody ratio such that the induction of dangerous hemolysis will occur. Finally, healthy donors do not usually have dangerous blood-group antibodies except, of course, for anti-A or anti-B. The Panel believes that testing of donor serum for unexpected antibodies using pooled cells is adequate. This subject is discussed further in the section on stored red cell products, testing of donor blood and antibody detection tests.

For testing of patients' bloods, the circumstances are significantly different. A transfusion of incompatible red blood cells into a patient who has even weak blood-group antibodies may lead to a serious hemolytic reaction. In addition, patients are far more liable to have blood group antibodies than are normal healthy blood donors; many of them have been transfused previously. Thus while pooled test red cells are acceptable for donor testing, they may or may not be acceptable for recipient testing. The Panel believes that the current practice, which allows the use of pooled cells for recipient serum screening, is safe provided tests are read microscopically, although perhaps not as sensitive as tests in which the cells are used individually. Studies are needed to clarify this significant problem.

(3) *As reagents for the identification of unexpected blood group antibodies.* When unexpected red cell antibodies are found in the serum of a patient, it is good practice to identify their specificities. This requires testing the patient serum with a panel of Reagent Red Blood Cells of known antigenic constitution. These red cell panels are prepared by individually packaging from 2 to 5 mL of diluted suspended cells obtained from each donor of a panel usually composed of 8 or 10 selected donors. The user is provided with information in the form of a graphic worksheet that lists the major antigens present or absent on the cells of each donor and a package insert with appropriate instructions.

Each manufacturer is required to establish a dating period beyond which the test red cell product cannot reasonably be expected to give satisfactory results. The dating period, from 21 days to 45 days after phlebotomy, is based upon data filed by each manufacturer with the Bureau of Biologics. The Panel believes that these products are, at times, used beyond the specified dating period and suggests that an appropriate caution against this practice be included in the package insert. The Panel has concerns about the stability of cell reactivity for periods of up to 45 days, in view of published data (Refs. 12 and 13) about the weakening of some antigens during storage. The Bureau of Biologics should review all current license applications to ensure that the data are adequate to support current dating periods for Reagent Red Blood Cells in view of these new studies.

Regulations specify that Reagent Red Blood Cells must have a cell concentration of no less than 2 percent, but specify no upper limit of concentration. It is common practice to provide this product with cell concentrations of approximately 5 percent. Some users, however, prefer to use a more dilute suspension of cells and routinely dilute the product before use. Because of this, the Panel believes that manufacturers should include instructions about how to dilute these test cells before use and the expected useful life of such diluted cells.

d. *Safety and effectiveness.* When shipped appropriately, stored and used as directed, and when the test results are interpreted properly, Reagent Red Blood Cells are safe and effective for all recommended indications.

e. *Labeling analysis.* Labeling of the vial and the package are appropriate. The package insert should be more specific about loss of reactivity with storage, should caution against use after the end of the dating period, and should indicate how additional information about the test cells can be obtained.

f. *Recommendations.* (1) Reagent Red Blood Cells (Human) should be placed in Category I.

(2) Appropriate studies are needed to evaluate whether pooled test red cells are adequate for antibody detection tests in patients.

(3) Package inserts should give specific instruction about how to obtain additional information about the test cells if it is needed.

(4) Manufacturers should provide information about how to dilute test cells if a lower cell concentration is desired and the useful life of the cells so diluted.

(5) The product name should be "Reagent Red Blood Cells."

5. *Anti-Human Serum*—a. *Introduction.* In 1908, C. Moreschi (Ref. 14) described the principal of a test that could be used to demonstrate the presence of antibody molecules on the surface of cells. Thirty-seven years later that test was rediscovered by Coombs, Mourant, and Race (Ref. 15) who used it to detect what were then known as blood-group blocking antibodies. These antibodies, found in the serum of some Rh-negative women, had been previously identified as the cause of hemolytic disease in Rh-positive newborn infants (Ref. 16). A year later Coombs and his coworkers used the same test to show that in some babies the disease was accompanied by an in vivo reaction between the infant's red blood cells and transplacentally acquired maternal antibodies. Later applications of the same test, now called the antiglobulin test, have included its use in the diagnosis of autoimmune hemolytic disease, the detection of drug-antibody-red cell interactions, the detection and identification of a multitude of blood-group antigens with nonagglutinating alloantibodies, the investigation of suspected transfusion reactions and pretransfusion compatibility testing. The antiglobulin test depends upon the fact that the reaction between red blood cells and some blood group antibodies leaves the cells coated with antibody protein (IgG), with various complement components, or with both, but does not result in agglutination or hemolysis. These red-cell bound proteins are best detected by the antiglobulin test in which the cells are reacted with Anti-Human Serum.

b. *Anti-Human Serum.* Rabbits or other animals such as goats injected with human plasma proteins produce antibodies against these proteins. The injection of human globulin leads to the production of anti-human globulin. When suitably processed, this anti-human globulin serum becomes a reagent capable of reacting with the same categories of human globulins that were present in the original immunizing material. Since a significant proportion of the human globulins are antibodies of the immunoglobulin G class, the reagent is a useful tool for detection of human IgG antibody. IgG molecules that have coated human red cells without causing agglutination will react with the anti-human globulin serum and cause visible agglutination of the antibody-coated red cells. As mentioned previously, antibody coating alone is often not adequate to cause agglutination of the cells, but does alter the red cells sufficiently so that they are recognized as abnormal by the body and are rapidly destroyed.

The injection of almost any human protein into a suitable animal will bring about antibody production. In this way reagents such as anti-IgA, anti-IgM, anti-IgG and antibodies against the various components of the complement system can be produced.

c. *Complement.* As related to the use of antiglobulin reagents in transfusion practice, the salient points about the red blood cell-antibody-complement interaction are:

(1) Some blood group antibodies react with blood cells by binding complement to the cell membrane. The antibodies then elute from the red cells leaving them coated with complement components but very little antibody. In vivo such reactions lead to hemolysis. In vitro they can be detected only by techniques that are sensitive to the presence of complement coating. The complement component that is present when such reactions occur in vitro is initially C3 and eventually C3d.

(2) Antiglobulin reagents intended to detect such complement-coated red blood cells must contain adequate amounts of either anti-C3 or anti-C3d activity (Ref. 17).

(3) The plasma from all normal persons contains small amounts of an IgM cold-reacting antibody called the normal, or naturally occurring, cold autoagglutinin. The amount of this antibody usually present in normal serum does not cause hemolysis of red blood cells. It may, however, lead to complement activation and fixation of C3 and C4 to red blood cells (Ref. 18). Such reactions have no clinical importance. These "false positive" tests can be avoided by limiting the amount of anticomplement in antiglobulin reagents intended for routine blood bank work (Ref. 6).

(4) C3d coated red cells are found in some patients with hemolytic anemia and in some cases of hemolysis associated with drug hypersensitivity (Refs. 5, 18 and 19). In some of these patients the test for complement coating of the red cells is the only one that will be positive (Ref. 5). Thus, the antiglobulin test has diagnostic value besides its usefulness in blood transfusion serology.

(5) Clinically important IgM blood group antibodies that fix complement almost always agglutinate or hemolyze red blood cells as well (Ref. 20). In these cases direct detection of complement by the antiglobulin procedure is rarely, if ever, necessary to prove that an antibody-red cell interaction has occurred, although it may be helpful.

(6) Clinically important IgG blood group antibodies almost always bind directly to red blood cells. If adequate tests for the presence of IgG are done, tests for complement binding usually add nothing (Ref. 21). Occasional exceptions are known.

d. *Direct and indirect antiglobulin tests.* The antiglobulin test is used to demonstrate that red blood cells have reacted with immunoglobulin molecules and are coated by either antibodies or some component of the complement system. The red cell-antibody intereaction may take place while the red cells are circulating (in vivo) or it may occur as part of a laboratory test (in vitro) in which serum to be tested for antibodies is mixed with normal red blood cells which carry the antigen(s) in question.

The direct antiglobulin test detects coating in vivo. Red cells from the patient are washed to remove unbound plasma protein and then reacted with Anti-Human Serum. No intermediate steps are involved.

The indirect antiglobulin test is used to detect alloantibodies or autoantibodies in a patient's serum or plasma. In performing the indirect test, patient's serum is mixed with antigenically appropriate red blood cells and allowed to incubate, usually at 37°C, for from 5 to 120 minutes. The cells are then washed and tested with Anti-Human Serum.

When positive, either test shows that antibody and red cells have reacted together. In the case of the direct test, the reaction occurred in vivo; in the indirect test it occurred in vitro.

e. *Indications*—(1) *Direct antiglobulin test*—(a) *hemolytic disease of the newborn.* In Hemolytic disease of the newborn (also known as erythroblastosis fetalis), antibody produced by the mother crosses the placenta and reacts with the unborn infant's red blood cells. This can occur only if certain conditions are met.

(i) Maternal antibody must be directed against a blood group antigen present on the fetal red cells.

(ii) The maternal antibody must be of the class (IgG) that is readily secreted across the placenta.

When these conditions are met the blood group antibody reacts with the unborn infant's red blood cells and may destroy a variable number of these fetal red cells before the child is delivered. At birth, antibody coating of fetal red cells is evidenced by a positive direct antiglobulin test.

While hemolytic disease of the newborn can result from almost any blood group incompatibility, the cases that occur when an immunized Rh-negative mother delivers an Rh-positive infant are best know. In this disease the infant's red blood cells are always coated with maternally derived IgG antibodies; for that reason only, antibodies with anti-IgG specificity need be present in the Anti-Human Serum reagents used for antibody detection caused by Rh incompatibility. The findings are identical for almost all cases of hemolytic disease of the newborn caused by other antibodies. A very few cases have been reported in which the antibodies are detected by anticomplement reagents but not by anti-IgG (Refs. 22 through 24). Such cases are rare, have been reported only from outside the United States, and were tested only by the less sensitive slide method which is not used much in the United States.

In hemolytic disease of the newborn caused by materno-fetal ABO incompatibility, the mother is usually type O and the child type A or B. In this disease, reactions with some United States licensed Anti-Human Serum reagents are negative, while others are positive. Reasons for these differences are obscure (Refs. 25 and 26).

Anti-Human Serum is an effective reagent when used for the diagnosis of hemolytic disease of the newborn. Lebeling of the reagent should indicate that a negative test does not always rule out the disease, especially when ABO incompatibility is suspected. Anti-Human Serum reagents containing antibodies in addition to anti-IgG are not necessary for this purpose, but can be used if the anti-IgG reactivity is adequate to detect IgG-coated cells using an accepted in vitro test system. Anti-Human Serums that do not contain anti-IgG activity are unsatisfactory for detecting infant red cell sensitization in hemolytic disease of the newborn and should be so labeled.

(b) *Autoimmune hemolytic disease.* The term autoimmune hemolytic disease (AHD) is often considered to be synonomous with autoimmune hemolytic anemia. Since all patients with AHD do not have anemia, the more general term will be used here. Patients with AHD can be classified as follows:

(i) *AHD warm antibody type.* This is a pathological condition in which circulating red blood cells react in vivo with autoantibodies. This reaction, at times in association with complement fixation, leads to variable rates of destruction of circulating red blood cells. Red cells are usually coated in vivo with IgG, rarely with IgA, and/or complement components. The responsible autoantibodies are produced by the patient himself/herself, and react with his/her own red blood cells. Normally a person does not produce antibodies that react with his/her own tissues. In approximately 80 percent of cases of AHD with significant hemolysin the direct antiglobulin test is positive with Anti-Human Serum containing only anti-IgG. In some cases, only complement components, especially C3d, are present on the red blood cells; in other patients both IgG and C3d can be detected (Ref. 27). The pattern of coating protein, i.e., IgG alone, C3d alone, or both, may be of diagnostic value (Ref. 28).

Use of Anti-Human Serum and the direct antiglobulin test is indicated in patients suspected of having AHD of the warm antibody type. An Anti-Human Serum reagent must contain a mixture of anti-C3d and anti-IgG, each in adequate amounts, to be satisfactory for this purpose. For a more definitive diagnosis, separate reagents, one pure anti-IgG and the other without anti-IgG but with adequate anti-C3d activity, are needed.

(ii) *Autoimmune hemolytic disease, cold antibody type.* This disorder is a pathological condition in which circulating red blood cells react in vivo with autoantibodies that react as cold agglutinins in vitro (Ref. 27). The autoantibodies involved are almost always IgM and are characterized by optimum reactivity at temperatures lower than 37° C (Ref. 28). In vivo, therefore, reactions between red cell and cold reactive autoantibodies probably occur predominately in areas of the body where temperature is lowest, for example, finger tips, skin, etc. where temperatures may reach 30–32 °C. These reactions invariably involve red blood cells, autoantibodies, and complement (Ref. 5). As the red blood cells, now coated with both antibodies and complement, circulate through warmer areas of the body, some are taken up by reticuloendothelial tissues and hemolyzed. Others have the unstable bond between the antibodies and red cells broken. This leaves circulating red blood cells coated with C3 which eventually converts into the C3d component of complement (Ref. 29). Because most red cells become coated with C3d by this mechanism, the direct antiglobulin test will be positive only if the Anti-Human Serum contains anti-C3d activity. Reagents intended for screening purposes must contain anti-C3d with or without anti-IgG. Reagents for definitive diagnostic purposes should be monospecific, that is, contain, anticomplement activity, particularly anti-C3d, and no anti-IgG. If anti-C4 is present, the amount should be such that reactions with normal red cells stored in the refrigerator as clotted samples will

be negative. Red cells stored in this way may become sensitized with C components by the small amount of so-called normal cold agglutin present in most samples of human serum.

(iii) *Autoimmune hemolytic disease, drug induced.* Autoimmune hemolytic disease, with or without anemia, is a relatively uncommon complication of therapy with many drugs in common use. Mechanisms by which drugs bring about the autoimmune state include (Ref. 18): Immune complex adsorption to circulating red blood cells; adsorption of drug to red cells followed by in vivo reaction of anti-drug antibodies with drug-coated red blood cells; drug-induced alterations of the red cell membrane causing it to adsorb plasma proteins, including IgG; unknown mechanisms which, in some cases lead to serological findings indistinguishable from AHD, warm antibody type. The direct antiglobulin test is essential for diagnosis of these drug-induced diseases and, as in other types of AHD, the Anti-Human Serum used for screening purposes must contain both anti-IgG and anticomplement activity (Ref. 18). While IgG is the protein most often found in drug-induced AHD, cases are known in which complement occurs either alone or in combination with IgG. A reagents containing a mixture of both anti-IgG and anti-C3d is adequate for screening, but IgG and C3d monospecific reagent are needed for optimum testing in these cases.

(c) *Evaluation of suspected incompatible transfusions.* The infusion of incompatible blood is a serious and occasionally fatal complication of transfusion therapy. Incompatible red cells react promptly with circulating antibodies and either are destroyed immediately in the vascular system or hemolyzed more slowly by the body's reticuloendothelial tissues. In the latter case, antibody-coated red cells may circulate for a variable time before they are destroyed and can be detected in the recipients since they cause a positive reaction in the direct antiglobulin test. This is called a "mixed field" reaction, which is a mixture of agglutinated incompatible donor red cells and unagglutinated recipient cells. The evaluation of suspected incompatible transfusions is an accepted and valid indication for the use of Anti-Human Serum. Reagents for this purpose must contain anti-IgG activity either alone or with anticomplement. Pure anticomplement reagents are unsatisfactory.

(2) *Indirect antiglobulin test.* When red blood cells are incubated in plasma or serum which contain antibody, a reaction will occur if the red cells contain an antigen against which the antibodies are directed. The cells, now coated with antibody, can be detected by any of several methods. The use of Anti-Human Serum is among the best of these, particularly for IgG antibodies that do not bring about agglutination or hemolysis of red cells after the original in vitro reaction (Ref. 30).

(a) *Antibody detection tests (antibody screening).* Serum from patients or blood donors can be tested for blood-group antibodies by mixing their serum with normal red cells selected for their antigen content and then testing these cells with Anti-Human Serum. The red blood cells used cannot possibly bear all of the known blood group antigens, but must possess those antigens considered to be most dangerous to potential blood recipients whose serums contain the corresponding antibodies. To achieve this goal usually requires testing a serum against two or three selected type O red cells, either individually or pooled. The exact techniques employed, for example, the ratio of serum to cells, the incubation time, the incubation medium (saline, normal serum, albumin, etc.) influence the sensitivity of the test. After incubation, the cells are washed and tested with Anti-Human Serum to see if an antigen-antibody reaction had occurred. A positive result indicates that the serum being tested contains blood group antibodies. The specificity of the detected antibody can be established by another series of tests.

Nearly all blood group alloantibodies that require the use of Anti-Human Serum for detection require only a reagent with anti-IgG activity. Such blood-group alloantibodies sometimes also lead to complement coating of the red blood cells, but in nearly all instances the IgG antibody itself is also bound to the cell, usually in greater quantity and more readily detectable than the complement. Occasionally, antibodies that are detected by anticomplement but not anti-IgG have been reported (Refs. 22 through 24 and 31 through 37). However, such antibodies must be very rare. Abelson and Wurzel (Ref. 38) in a communication to this Panel report studies of 4,980 specimens. They found no reactions with "broad spectrum reagents" which were not also found with anti-IgG. Beck and Marsh (Ref. 39) report a combined experience of patient transfusion problems from 500 metropolitan New York hospitals along with blood donor antibody testing on more than 5.5 million units. In no case was a blood-group alloantibody detected solely because of its complement-binding capability. Petz (Ref. 40) has tested 113 sera selected because they contained antibodies with presumed complement-binding ability. Of this group, there were only one specimen, an anti-S, in which the antibody could be detected by anticomplement alone. Applying these statistics to usual transfusion practice, Petz estimates that the largest hospital in the San Francisco Bay area can expect to find such an antibody only once in 12.5 years, while the average-sized hospital will encounter such antibodies only once in 107 years.

While neither of these studies has shown the need for anticomplement activity, the results are preliminary and must be interpreted in light of the more than 20 years of experience with the antiglobulin test. An early report by Millison and Cutbush (Ref. 36) pointed out that to cause a positive antiglobulin reaction, some alloantibodies required an Anti-Human Serum with anticomplement activity. Reports from Stratton's laboratory (Refs. 24, 31 and 33), as mentioned earlier, suggest an important role for anticomplement as component of Anti-Human Serum. Pollack, Reiss and Treacy (Ref. 37) showed that in some cases a large amount of complement was attached to a red cell surface that was only weakly sensitized with IgG. Pollack (Ref. 41) has reported to this Panel that he has studied alloantibodies which caused severe transfusion reactions which were detected only by anticomplement. Finally, Sherwood, Haynes and Rosse (Ref. 34) have reported a hemolytic transfusion reaction caused by the failure of commercial antiglobulin reagent to detect complement. These reports document cases in which antibodies were detected by reagents containing anticomplement but not by anti-IgG alone. While cases where only complement is present on red cells are infrequent, there is little doubt that some blood group antibodies both bind to red cells and fix complement. In these cases, the amount of red cell-bound protein detected by a reagent containing both anti-IgG and anticomplement must be greater than the amount detected by either antiserum alone. Hence, broad spectrum reagents can be expected at times to yield stronger reactions which are easier to interpret that either anti-IgG or anticomplement alone. This appears to be a reason to require the use of reagents with boty specificities, but data to support this contention are inadequate. For the present, the Panel believes that reagents lacking antibodies against complement may be used for detection of red cell alloantibodies provided the package

insert warns that some antibodies may not be detected optimally. A multcenter study is underway to evaluate the requirement for anticomplement activity in the Anti-Human Serum used for this indication. This study should also evaluate the frequency and nature of the problems ("false positive") associated with reagents containing anticomplement.

(b) *Compatibility testing (crossmatching).* Compatibility testing, also known as crossmatching, is done before transfusion to ensure that the red blood cells intended for transfusion will be compatible. The usual procedure (Ref. 42) involves mixing the red blood cells of the intended donor with the serum of the recipient. After incubation the mixture is washed and Anti-Human Serum is added. The procedure is identical to that used for antibody detection tests except that cells of the intended donor replace the selected screening cells used in the latter procedure. Since both antibody detection testing and compatibility testing are identical procedures except for the choice of cells, similar considerations apply to both.

The Panel concludes that Anti-Human Globulin Serum is appropriately used in the indirect antiglobulin procedure as a part of donor-recipient compatibility testing. Anti-IgG is essential in this test. Until more definitive data are available to the contrary, the Panel concludes that additional anticomplement activity is not required. If antiglobulin serum contains anticomplement it must have adequate ant-C3 or C3d. Anticomplement (especially ant-C4), if present, should not be in such amounts that false-positive reactions occur. If antibodies to C3 or C3d are absent in a reagent serum, the package insert should state that some blood group alloantibodies may not be optimally detected. These recommendations are the same as those for the use of Anti-Human Serum in the antibody detection procedure.

(c) *Detection of D^u.* The presence of absence or the D blood group antigen of the Rh blood group system is important since the differentiation of donors and recipients into Rh-positive and Rh-negative groups is based on the presence or absence of this one Rh factor. Some variants of the Rh antigen, termed D^u, react weakly or not at all when tested with usual procedures (Ref. 43). Despite these weak reactions these bloods are antigenically Rh positive and, if transfused into Rh negative recipients with high titered anti-Rh antibodies, could cause serious reactions. The D^u antigen can be detected by several techniques, one of which involves the use of Anti-Human Serum. Cells to be tested are incubated with a reagent antiserum specific for the D blood group antigen. The cells, if D^u positive, react with and become coated by the anti-Rh but are not agglutinated. The addition of Anti-Human Serum after the cells are washed agglutinates these cells, thus showing them to be D^u positive.

The Panel concludes that Anti-Human Serum is indicated for use in the test for D^u. The reagent needs to contain only anti-IgG for this purpose since all anti-Rh reagent antiserums used for D^u testing are of IgG class and do not fix complement.

(d) *Red blood cell typing.* Red-cell typing is done by mixing the cell to be tested with antiserums of known specificity and examining the mixture for evidence of a reaction. In many cases, the antiserums used do not agglutinate the red cells directly, and the fact that an antigen-antibody reaction has occurred (a positive test) can be determine most efficiently using the indirect antiglobulin test. This type of testing is required for many of the reagents now licensed for red-cell typing. These include anti-Fy^a serum, anti-K serum, anti-Lu^a serum, and many others. The Anti-Human Serum used for these tests needs to contain only anti-IgG activity since complement components are not ordinarily involved in these reactions. The use of Anti-Human Serum in the indirect antiglobulin test is appropriate in these tests.

f. *Labeling analysis.* The major portion of the labeling for Anti-Human Serum reagents is not on the package but in the package insert. This circular should provide the laboratory worker with sufficient information to use, store, and evaluate the product. For the sake of uniformity, package inserts should use similar terminology. For example, either of the terms "crossmatching" or "compatibility testing" are appropriate, but one should be selected as the proper one with appropriate synonyms in brackets. In a like manner, terms for the various immunoglobulins, complement components, and diseases (autoimmune hemolytic anemia versus acquired hemolytic anemia versus autoimmune hemolytic disease) should be standardized.

A paragraph containing information about the specific antiglobulin activities present or absent in the reagent should be included. Methods used to determine these activities should be given in enough detail to allow the user to evaluate the product if necessary. In addition, the vial label should give specific information about the antibody activities included in the product.

Detailed instructions for the use of the product should be included. Information about how to set up necessary controls is essential.

g. *Summary.* Anti-Human Serum is a valuable reagent for blood bank work. The Panel recommends that it be placed in Category I. The use of Anti-Human Globulin Serum is indicated for:

Direct antiglobulin test.

(1) Diagnosis of hemolytic disease of the newborn.

(2) Diagnosis of autoimmune hemolytic disease.

(3) Evaluation of suspected incompatible transfusion reactions.

Indirect antiglobulin test.

(1) Antibody detection.

(2) Compatibility testing.

(3) Detection of D^u.

(4) Red blood cell typing.

Anti-Human Serum may contain antibodies against any of the human plasma proteins; however, reagents for use in blood banks, transfusion services, or laboratories involved in the diagnosis of immunohematologic disorders should contain antibodies specific for immunoglobulin G and selected components of the complement system. These may be present as individual components or may be mixed to produce Anti-Human Serum that has more than a single reactivity. Anti-Human Serum intended to screen patients for autoimmune hemolytic disease must contain activity against IgG and the C3d component of complement. More definitive characterization of these disorders requires reagents with either pure anti-IgG or pure anti-C3d activity. Reagents for all other indications must contain anti-IgG activity and may also have anti-C3 or C3d activity. In all cases the labeling must specify the antibody activities present, how tests for this were done, and the indications for which the reagent is suitable. Sufficient information must be included to allow the user to test the reagent to determine which antibody activities are present.

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20. Hepatitis B Testing

1. *Introduction.* Post-transfusion hepatitis has long been a serious medical problem. In 1970 the National Research Council estimated that there were approximately 30,000 cases of clinical post-transfusion hepatitis annually with a mortality rate of approximately 10 percent (Ref. 1). It was also estimated that there may be up to five times that many cases that are not clinically detectable. Other studies (Ref. 2) estimated the risk at 6.4 cases per 1,000 units of blood transfused, with one-third of these being icteric, but more recent studies suggest a higher risk, of between 7 and 10 percent of all blood recipients (Ref. 20).

The discovery of the hapatitis B surface antigen (HBsAg), previously called Australia antigen (Au) or hepatitis-associated antigen (HAA) was a major scientific breakthrough toward characterizing the hepatitis B virus and decreasing the incidence of post-transfusion hepatitis (Refs. 3 and 4). HBsAg, which is thought to be the outer coat or surface protein of the hepatitis B virus, is released into serum in three major morphologic forms available on electronmicroscopy: as a 20-nm sphere, as an elongated tubular form, and as an outer coat component fragment of the complex 42-nm Dane particle (Refs. 5 and 6). The presence in serum of HBsAg and studies of transfusion hepatitis have demonstrated an association between HBsAg and infectivity (Refs. 7 and 8). Testing all donor blood for HBsAg was mandated the Bureau of Biologics in 1972 (Ref. 9) and has resulted in a significant reduction of hepatitis B related post-transfusion hepatitis (Refs. 8 and 10). Other forms of hepatitis, including hepatitis A, but particularly the group of so-called non-A, non-B hepatitis now comprise the majority of post-transfusion cases (Refs. 12 and 13). However, there continue to occur cases

of type B post-transfusion hepatitis caused by blood found to be negative by the most sensitive tests currently available. New tests for other markers of hepatitis B infectivity, such as detection of antibody to the hepatitis B core antigen (HBcAg) may permit detection of potentially infective donor blood in some of these cases (Ref. 14).

Test methods for HBsAg have improved rapidly. HBsAg was initially detected by agar gel diffusion, a test of low sensitivity requiring a long incubation time. When HBsAg testing of blood donors became mandatory in 1972, second generation tests were required, that is, tests equal to or greater in sensitivity than counter-immunoelectrophoresis. These tests were more rapid to perform and had greater sensitivity than agar gel diffusion. In 1974, this category of tests was superseded by third-generation tests which are more reliable and reported to be 50 to 1,000 times more sensitive than counter-electrophoresis. Current third generation tests include radioimmunoassay (RIA), reverse passive hemagglutination (RPHA), and ezyme-linked immune adsorbant assay (ELISA).

As set forth by the Bureau of Biologics, third generation tests for HBsAg are defined by the ability to detect certain HBsAg-positive serum samples in a Bureau of Biologics reference panel. This panel is comprised of HBsAg positive and negative samples and includes samples with greater and lesser amounts of the antigen. Both antigen subtypes, adw and ayw, are included. The reference panel also includes a variety of HBsAg positive specimens variously diluted, both to the current limits of detectability by available tests and to dilutions that are not detectable by any currently available method. New tests that may be introduced, must perform acceptably against this Bureau of Biologics' serum panel.

2. *Test methods*—a. *Radioimmunoassay tests (RIA).* Polyethylene tubes or another suitable "solid-phase" carrier such as a bead supplied by the manufacturer are coated with specific anti-HBsAg. When serum containing HBsAg is added to the anti-HBsAg coated "solid-phase," the HBsAg will "attach" to the antibody. Excess serum is removed, and the solid-phase complex is washed, and radiolabeled anti-HBsAg is added. The anti-HBsAg becomes fixed to the "bound" HBsAg forming a (solid-phase) antibody antigen-^{125}I-labeled antibody "sandwich". The radiolabeled antibody remains in the tube or on the "solid-phase" after a series of washes, producing a strongly radioactive tube or "solid-phase". A serum nonreactive for HBsAg will not provide a site for binding of the readioactive antibody. Radioactive anti-HBsAg is therefore removed by the subsequent washes, resulting in a test with low radioactivity.

RIA tests results are determined by counting radioactive emissions in a gamma counter and comparing test samples with control standards. Reading of this test is thus objective.

b. *Reversed passive hemagglutination tests (RPHA).* Reagent human erythrocytes are coated with specific antibody (anti-HBsAg) and stabilized by lyophilization. In the presence of HBsAg, the antibody-coated erythrocytes form a complex leading to a visible hemagglutination. If the antibody coatd cells are not agglutinated because HBsAg is absent or there is insufficient HBsAg in the sample, the antibody-coated erythrocytes settle in a recognizable nonagglutinated pattern, a sharp compact button. The interpretation of this test is subjective; it requires distinguishing agglutinated-positive tests from unagglutinated-negative results.

c. *Enzyme-linked immune absorbent assay (ELISA).* This test is similar in principle to "solid-phase" RIA tests except that enzyme-linked reagent antibodies of anti-HBsAg specificity are employed at the final stage to form a solid-phase antibody-antigen-enzyme-linked antibody "sandwich."

Instead of direct transfer to a gamma counter, a colorless enzyme substrate, *p*-nitrophenyl, is added to the "sandwich." Hydrolysis of the colorless substrate by the enzyme linked to the anti-HBsAg forms a yellow end product. The intensity of the color, measured spectrophotometrically, defines the presence of HBsAg when appropriate controls are employed. Interpretation of this test is also objective.

3. *Antibody used for testing.* Antibodies used for the detection of HBsAg in third generation tests vary considerably among the current licensed procedure. Antibodies raised in a variety of animals immunized with HBsAg as well as antibodies from humans are used. These are summarized in Table 1 below. As all test procedures have a significant incidence of false-positive reactions, some due to the presence of heterospecific antibodies, all manufacturers recommend a neutralization test for confirmation of specificity. For confirmatory testing, all manufacturers provide human anti-HBsAg (with one exception where chimpanzee anti-HBsAg is used) to neutralize specifically the presumed antigen prior to the perfomance of the confirmatory detection test, which should become negative. Antibodies in all test procedures must also contain specificities to the two major subclasses of HBsAg, ay and ad.

In some cases manufacturers offer alternative test procedures employing different temperatures of incubation to shorten the time required for the test. These are to be used when the usual time required for performing the test is too great, and the need for the particular blood product is immediate. Short incubation time tests are usually less sensitive than tests performed in the standard manner with material from the same manufacturer.

TABLE 1.—SOURCE OF ANTIBODY FOR LICENSED TESTS FOR HBSAG

	Bound to solid phase	Label	Neutralization
RIA			
RIA International	Goat	Human	
Electronucleonics	do	Goat	Human.
Abbot	Guinea pig	Human	Do.
Hyland	do	Chimpanzee	Do.
RPAH			
Abbott	do	do	Do.
Ortho	Chimpanzee	do	Chimpanzee.
ELISA			
Cordis	Horse	Horse	

4. *Test sensitivity and performance.* Direct Comparisons of test sensitivity between RIA and RPHA have been made. In one study (Ref. 15), when comparisons were made by parallel testing of serial dilutions of HBsAG positive samples, RIA was able to detect one-fourth the quantity of HBsAg subtype ad and one-sixteenth the quantity of a subtype ag antigens when compared with RPHA. Another study, performed by the Bureau of Biologics and shown in Table 2 below, also indicates that RIA, in general, is more sensitive than RPHA. However, a third study (Ref. 16) indicates that the sensitivity of RPHA is comparable to RIA.

TABLE 2.—COMPARATIVE SENSITIVITIES OF LICENSED THIRD GENERATION TESTS*

HBsAg Sample No. (subtype)	RIA-1	RIA-2	RPHA-1	ELISA-1	RPHA-2
4 (ay)	32	16	16	8	16
12 (ay)	32,768	16,384	16,384	,096	8,192
19 (ay)	2,048	2,048	512	512	512

TABLE 2.—COMPARATIVE SENSITIVITIES OF LICENSED THIRD GENERATION TESTS*—Continued

HBsAg Sample No. (subtype)	RIA-1	RIA-2	RPHA-1	ELISA-1	RPHA-2
37 (ay)	64	64	64	16	32
6 (ad)	8,192	8,192	8,192	2,048	2,048
18 (ad)	128	64	128	32	32
20 (ad)	2,048	1,024	1,024	256	512
27 (ad)	16,384	16,384	8,384	8,192	4,096
32 (ad)	128	128	128	64	64
33 (ad)			64	64	64

*End-point dilution titers.

When comparing RIA and RPHA in their ability to detect HBsAg positive samples in populations containing antigen positive and negative individuals, RIA testing results in a slightly higher detection rate. Some of the published data are summarized in Table 3 below.

TABLE 3

No. tested	No. RPHA positive	No. RIS positive	(Reference)
7,067	34	34	(17)
3,186	5	6	(16)
25,357	173	176	(15)

Proficiency testing indicates superior performance of RIA when compared with RPHA in most cases. The AABB-CAP survey (Ref. 19), including eight proficiency test panels distributed over a 3-year period gave the following results: The percentage of correctly identified positive samples by RIA was between 98.5 and 99.8; by RPHA it was between 79.5 and 98.1. The differences between the two test methods was magnified if only weakly reactive positive samples were compared. This study also reported that in panels of HBsAg positive and negative samples, 87.8 percent of participants correctly identified all samples by RIA but only 55.8 percent by RPHA.

In two more recent AABB-CAP proficiency test panels studied in February 1977 and February 1978, RIA also gave higher detection rates and correct response rates than did RPHA. These data can be summarized as follows:

1977 (AABB-CAP PANEL)

	HBsAg Pos. (15), number of correct responses/total responses	Percent	HBsAg Neg. (5), number of correct responses/total responses	Percent
RIA	5243/5493	95.5	1776/1845	96.3
RPHA	829/961	86.3	315/320	98.4

1978 (AABB-CAP PANEL)

	HBsAg Pos. (7), number of correct responses/total responses	Percent	HBsAg Neg. (3), number of correct responses/total responses	Percent
RIA	3179/3207	99.1	1293/1317	98.2
RPHA	497/519	95.8	211/220	95.9

Proficiency tests sponsored by the Centers for Disease Control (CDC) gave inconsistent results. In the first CDC Panel (Diagnostic Immunology 1977 I, Hepatitis B, September 1977), RIA gave a 99-percent correct response rate as compared with RPHA which had a 93-percent correct response rate. In the second CDC Panel (November 1977), RIA and RPHA had equally good performance, both 99-percent correct, but RIA showed a higher false-positive rate.

Evaluations of ELISA in comparison with RIA and RPHA have been few. In one study, in titration of HBsAg positive samples, ELISA gave results comparable to RIA (Ref. 19). Titration studies performed by the Bureau of Biologics indicate ELISA to be less sensitive than RIA but comparable to RPHA (Table 2). In field tests, ELISA also gave results comparable to other third-generation tests, although the type of tests is not identified (Ref. 19). ELISA seems to be associated with a relatively high incidence of false-positive results (Ref. 19).

These data must be considered with caution. As there are a number of manufacturers of both RIA and RPHA test kits, it is possible that not all RIA or RPHA products are of equal sensitivity or performance. Although the currently available data are few, there is reason to believe this is correct. Thus, comparisons of tests on a generic basis, e.g., RIA versus RPHA, may not be an entirely valid basis for drawing conclusions with regard to test efficacy. Finally, it must be appreciated that all data agree that any third-generation test is far superior to second-generation tests for screening blood donors for HBsAg.

7. *Summary.* a. Third-generation tests for the detection of HBsAg in blood donors are far more sensitive and proficient than second-generation tests, and their mandated use has reduced the occurrence of hepatitis B in blood recipients.

b. Among third-generation tests, RIA is most sensitive for the detection of small amounts of HBsAg. There are no data that relate such sensitivity to possible infectivity.

c. In general, proficiency testing has demonstrated that RIA detects more HBsAg positive samples than other third generation tests.

d. All currently available licensed third-generation tests meet minimal standards for safety and efficacy.

e. There is some evidence that among RIA or RPHA tests there may be some variability in sensitivity and performance.

f. There is evidence that short-incubation RIA is less sensitive than overnight incubation RIA.

8. *Recommendations.* a. The licensed third-generation hepatitis testing reagents are recommended to be placed in Category I.

b. A workshop should be organized to attempt to redefine third generation tests on the basis of an absolute minimal standard amount of HBsAg that must be detected by any method. All tests must be able to detect this minimal quantity consistently without an inordinate number of false-positive results, in order to be designated a third generation test.

c. Studies relating test sensitivity with infectivity should be encouraged.

d. The standard should be reviewed periodically to reflect new advances in the field which might result in tests of even greater sensitivity.

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21. Antivenins

Generic Statement and Product Reviews

1. *Introduction*. This statement reviews the licensed antivenins and the associated horse serum control materials. The products are Antivenin (*Crotalidae*) Polyvalent and Antivenin (*Micrurus fulvius*) manufactured by Wyeth Laboratories, Inc., and Antivenin (*Latrodectus mactans*) manufactured by Merck Sharp and Dohme.

Therapeutic antivenins are the fractionated globulins from the serum of horses which have been immunized with a clinically important toxic venom. Three major preparations are available: (1) Polyvalent antivenin, (*Crotalidae*), the North American pit vipers, (2) antivenin (*Micrurus fulvius*), the North American Coral Snake, and (3) antivenin (*Latrodectus mactans*), the Black Widow Spider. These antivenins have a long history of development and clinical use and have well-established indications for treatment of the respective envenomations, which include toxic bites of most of the significant indigenous North American animals from which life may be at risk.

It is of note that many toxic animals from throughout the world have been imported into the United States into zoos, research institutes, and, in some instances, private collections. Occasional accidental bites from these animals constitute a small but serious health problem for the involved individuals. A volunteer organization has developed a network of information centers and repositories for antivenins appropriate to these exotic toxic animals, situated through the United States. These materials come from primarily foreign origins and are imported into the United States under individual IND exemptions at the present time, since their distribution is not regarded as primarily commercial in nature.

Most animal venoms are complex mixtures of proteins and peptides that have a wide variety of biochemical and physiological activities which result in their toxic effects (Refs. 25 and 26). In the case of the venom of *Crotalidae*, several of the venom fractions have primarily local effects, resulting in inflammation, tissue necrosis, and vascular thrombosis. Other fractions have more general systemic effects, particularly the so-called "lethal fraction" which is primarily responsible for death of man or test animals. Other prominent systemic effects are induction of hemolysis and activation of the blood coagulation system (Refs. 3 through 10).

This discussion will not include comprehensive consideration of the management of toxic bites, but will focus on the use of antivenins. It is important to recognize the need for comprehensive understanding of the problems prior to treatment of such a patient.

Antivenin is standardized by a biological test in mice for its protective effect primarily against the lethal factor. The components of these venoms which act locally may be variably neutralized by antivenin. Local injury may already have been produced by the time venom neutralization is attempted; and the antivenins themselves are not standardized for their action against these locally injurious components. Thus, it should be recognized that the *Crotalidae* polyvalent antivenin is a useful therapeutic agent primarily for its life-saving effect and that local injuries at the site of a bite may be quite severe even in face of adequate antivenin administration. It is important that the total management of these bites is understood by the person responsible for their treatment and that actions not be taken, such as excessive tissue cooling, obstruction of the arterial circulation, or widespread incision which may well increase the local tissue damage without any therapeutic effect; corticosteroid therapy should not be employed except on very specific indications. By contrast, the venoms of the North American Coral Snake and the Widow Spiders act predominantly against the central nervous system, frequently with little or no local reaction which predicts the degree of envenomation. The first symptoms suggesting that envenomation has occurred may be rapidly developing paralysis, including respiratory paralysis. The administration of antivenin in this situation is quite specific, but again it must be integrated into a program of management which includes support of the patient, with maintenance of adequate respiratory function and oxygen uptake (Refs. 11 through 23).

A wide variety of other systemic effects occur primarily in association with bites of pit vipers and to a lesser extent coral snake envenomations. Many of these appear to be related to activation of the coagulation system with associated fibrinolysis, thrombocytopenia, a variety of hemorrhagic manifestations, hemolysis and impairment of renal function. Maintenance of adequate hydration, blood pressure, and urinary output during recovery from a bite is important for successful outcome. These effects are relatively short lived and commonly begin to correct themselves following

adequate treatment with antivenin during the first 48 to 72 hours of treatment. In some instances, specific interventions such as red cell transfusions or correction of severe hemostatic defects may be indicated on the merits of the specific problem itself (Ref. 2).

a. *Nature and sources of immunizing antigens.* A number of problems exist with regard to the sources and standardization of the immunizing antigens used in the preparation of antivenins. As noted previously, venoms are a complex mixture of materials with wide range of biochemical effects primarily due to their enzymatic activities. There appear to be variations between species and subspecies in the quantitative and qualitative characteristics of the venoms. There are also variations within a species over different geographic ranges and seasonal variability as well. There may well be variability in the venom obtained from a single individual animal depending upon the conditions under which the animal is kept, the frequency of venom removal and other factors not well understood (Refs. 12 and 16).

In general there is not a serious problem of supply of snake venoms since these are available commercially from a number of serpentariums. This, however, does not eliminate the kinds of variability just mentioned.

In the case of the venoms of the widow spiders, the problem is more serious. It should be noted that in most instances the venom is not a secreted product of the animals, but rather an extract of the removed venom sacs of a large number of individual animals. Thus it will contain materials derived from the venom producing apparatus in addition to the venoms themselves. There have been significant problems in obtaining reliable supplies of standardized venoms for the widow spiders. It is probable that little of the currently available antigenic venom is actually derived from the Black Widow Spider itself; rather it is derived from a variety of related spiders from both North and South America, the venom of which has a high degree of cross-reactivity with that of the Black Widow Spider. Since the antivenin produced with these antigens is useful in the treatment of toxic bites from this group of related spiders, it seems reasonable to rename the material to reflect this fact (Ref. 27).

Not only must venoms be obtained for purposes of immunization of horses, but they must also be available in standardized and stable form for subsequent testing of the potency of the antibody that has been produced. This must be done both by immunochemical means and more importantly by in vivo mouse protective tests conducted by both the manufacturer and by the Bureau of Biologics which also tests the material for potency before release. Thus the secretion, procurement, storage and stability of the antigenic venoms is a crucial problem in the production of these materials. At the present time, the situation with regard to sources of appropriate venoms seems adequate, but one might easily envision situations in which particularly the venoms of the widow spider may be in very short supply.

b. *Antivenins prepared in other species.* Because the products reviewed here are prepared in horses, their principle toxicity relates to horse serum sensitivity of the recipient. This has lead to a number of efforts to develop antivenins in other species, particularly in goats, so that at least small supplies may be made available to patients who are highly sensitive to horse serum, possibly as a result of previous treatment with antivenin. These efforts have not been commercially supported and at the present time there is no such alternative source of antivenin available in the United States. Nevertheless there remains a need for such products. Attention should be given to a variety of ways in which they might be produced, distributed, and utilized for the treatment of these seriously compromised patients in whom desensitization to horse serum may be very hazardous, particularly if it is not carried out by someone thoroughly familiar with the procedure and who has adequate monitoring capability and understanding of the management of anaphylaxis (Ref. 24).

2. *Indications.* The primary indication for administration of antivenin is envenomation as the result of a bite. This deceptively simple criterion has many difficulties in its application. First, as many as 20 percent of toxic snake bites may not be accompanied by the injection of venom (Refs. 3 and 4). Thus, the presence of a bite alone does not provide an indication for the administration of antivenin. In the case of pit viper bites, usually local reactions promptly signal the occurrence of envenomation. Under some circumstances where venom is injected deeply into muscle or into a vascular structure, local reaction may be minimal or absent, and the first indications of envenomation are systemic symptoms. Furthermore, it is important to estimate the degree of envenomation in order to administer an adequate amount of antivenin.

Close observation of the patient and the site of the bite with periodic revisions of the estimate of the degree of envenomation and reassessment of the amount of antivenin administered are thus indicated in the management of these injuries. In general, underestimation of the degree of envenomation is more hazardous than overestimation, although maximum treatment with antivenin in every case cannot be justified on this basis.

A number of grading systems for evaluating the degree of envenomation have been employed in the past. These systems may require a period of up to 12 hours to permit accurate evaluation. This is of course well beyond the period of up to 4 hours when antivenin has its maximum therapeutic effectiveness. These delayed grading systems of the amount and/or severity of envenomation may have utility in predicting or evaluating ultimate clinical outcomes, or in clinical investigation, but they should not be employed in labeling as a guide to therapeutic antivenin administration and dosage. Emphasis must be given to the necessity of an early clincial judgment about degree of envenomation as the basis for prompt administration of antivenin. Labeling material should reflect this position.

The bite of the Coral Snake, as noted previously, may be associated with no local symptomatology other than the superficial injury of the bite itself. In these patients, very close attention to clinical manifestations are necessary to judge the degree of envenomation and the appropriate amount of antivenin for administration (Ref. 13). Widow spider bites can be regarded in the same way (Ref. 17).

It is of great importance that the exact identification of the animal responsible for the bite be made if possible. The probable severity of envenomation can be predicted to some degree both by the species and the size of the responsible animal. These judgments must of course be made in the light of the body size of the victim, with the recognition that equivalent amounts of envenomation are much more serious in children, particularly small children. For example, bites of the so-called "pygmy rattlesnake" are rarely lethal in adults but may be severe in small children. The same may be said of Copperhead and Water Moccasin bites. By contrast the venom of the Mojave rattlesnake (*C. scutulatus*) is extremely toxic (Ref. 2).

It should be recognized that the judgment required to estimate the degree of envenomation is not simply acquired, and the help of persons

experienced in the management of toxic bites should be sought where it is available. The always confused and sometime hysterical situation surrounding the management of the snake bite victim should not lead to precipitous and unwise intervention. Rather, a calm and thoughtful examination of the entire situation and an evaluation of the severity of the injury is always preferable to a hurried, routinized intervention. Although these are emergency situations, time is always available to make such an evaluation. It should be recognized that the unwarranted administration of antivenin to a patient, who because of occupational reasons or other factors affecting his/her exposure has a high likelihood of repeated bites, will certainly produce a serious problem if that patient is bitten subsequently. The decision to administer antivenin can be lifesaving and frequently is, but its consequence will almost certainly be a lifetime sensitivity in varying degree to horse serum and possibly related materials.

3. *Effectiveness.* A large volume of literature supports the effectiveness of antivenins in the management of these toxic bites. The principle limitations on effectiveness are the time interval from the occurrence of the bite to the administration of antivenin. Treatment within 4 hours of the bite once the presence of envenomation has been determined appears to be optimal, with declining effectiveness over the following 16 hours. In severe envenomation administration of antivenin has been recommended even if a 24 or more hour interval has occurred since the injury (Ref. 24).

A second limitation on effectiveness appears to be undertreatment. Present package sizes provide 10 mL of reconstituted dry antivenin as the therapeutic dosage unit. Since two to four vials of antivenin are recommended for even mild envenomation and 10 to 20 vials where severe envenomation has occurred, it might be reasonable to increase the individual package size by a factor of at least two.

4. *Safety.* The hazard associated with all of these antivenins is that of horse serum sensitivity of the recipient. Great care must be taken in determining the recipient's status from this point of view both by a carefully obtained history and by appropriate testing with horse serum. Carefully done intradermal testing is mandatory. Severely hypersensitive individuals may have severe reactions to even the small quantities of antigens in the intradermal test. Intradermal testing should not be done unless preparations have been made to manage anaphylaxis. A conjunctival test, whereas it may be of use in detecting severely hypersensitive people, also is subject to significant false positives unless it is very carefully carried out and supervised. The conjunctival test may provoke severe conjunctivitis in severely hypersensitive people, sometimes with threats to vision. Since an intradermal test should be done, there is little reason to carry out the conjunctival test.

Even in the absence of positive skin tests, an anaphylactic reaction may occur shortly after the administration of antivenin, and its immediate management with appropriate measures will be lifesaving. Delayed reactions of the serum sickness type generally occur within 5 to 25 days after administration in patients receiving horse serum for the first time and somewhat earlier in patients who have been immunized to this material previously.

Patients may be sensitive to horse serum in the absence of prior horse serum injections. Contact with horses, possibly by inhalation of horse dander and other exposures, appears to be capable of sensitizing susceptible individuals. In other instances, patients may be sensitive without any known or remembered contact with horses or horses proteins. The history given by patients who must receive horse serum products is thus of limited value. A valid history of prior horse serum administration should always be regarded as strong evidence of presumed hypersensitivity. The absence of such a history cannot be relied upon to indicate lack of sensitivity.

Hypersensitive patients will require desensitization prior to the administration of antivenin. This can be accomplished according to a standard dosage regimen in almost every patient, with little risk in experienced hands. The procedure does clearly have a significant associated risk when it must be carried out by people with inadequate experience or facilities to monitor and support the patient during desensitization. In general, the existence of hypersensitivity in the envenomated patient is not a contraindication for the administration of the antivenin, but it should be recognized that this situation increases the risk to the patient of antivenin treatment. The details of an adequate procedure for desensitization will not be discussed here, although the general procedure is considered in the labeling (package insert) provided with all of these products.

Antivenins should not be injected at the site of a bite, or distal to the wrist or ankle. They are maximally effective when delivered to the envenomated tissues by the circulation.

5. *Benefit/risk evaluation.* An abundant literature supports the position that the benefits of antivenin therapy, appropriately used, markedly outweight the risks involved.

6. *Recommendations.* a. These products are recommended to be placed in Category I.

b. Antivenins from alternative animal sources should be made available in the United States to treat patients who have severe horse serum hypersensitivity, particularly for patients who are at high risk of repeated bites.

c. Package dosage sizes should be increased by a factor of two.

7. *Labeling recommendations.* a. Easily recognizable common names should be included in the official name for the Crotalidae antivenin as is done for the Coral Snake antivenin. The addition of the words "Rattlesnake, Copperhead and Moccasin" is recommended.

b. Since it is likely that the antigen used for the preparation of the "Black Widow Spider Antivenin" is not actually derived from the Black Widow Spider but from closely related species, the name of this product should be changed to reflect this fact. An appropriate common name would be "Widow spider Spp. antivenin". These changes in title would have the purpose of improving the identification of the product for personnel with less training who may be called upon to handle them.

c. Antivenin should be administered intravenously, except in circumstances where this is not possible. Intramuscular administration results in delayed absorption and may well result in inadequate treatment, particularly in severe envenomations. At the persent time, intramuscular administration is still recommended in labeling. The labeling should be revised to remove the recommendation for intramuscular injection, except in circumstances where intravenous administration is impossible.

d. All references to delayed or deferred determination of the degree of envenomation as a basis for determining the appropriate dose of antivenin should be eliminated from labeling materials. Guidelines should be given for estimation of severity of the bite based upon initial observations and further observation over a short time period, an hour or two at most. Emphasis should be placed on prompt treatment, rather than precise calculation of the degree of envenomation.

e. The limited usefulness and possible hazard of the traditional conjunctival test for horse serum sensitivity should be made explicit in labeling materials. Emphasis should be placed upon proper performance of a skin test and its interpretation. Emphasis should be given to the possibility that anaphylaxis may occur after even a test dose of horse serum in severely hypersensitive patients.

f. Package inserts should be printed in more durable booklet form if possible and the material reorganized in such a fashion as to provide a primer on the management of victims of the specific toxic bit. The most important clinical material should be printed in somewhat larger type to provide inexperienced people with an easily comprehended guide to management of the envenomated patient. Package inserts of this type might have a lasting value as readily available instructional materials in emergency rooms, isolated aid stations, and other places where bitten patients are treated. The assistance of experienced clinicians in this field should be sought in the reorganization and preparation of this material.

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22. Normal Horse Serum

Generic Statement

Normal Horse Serum, a 1:10 dilution of sterile unfractionated normal horse serum is employed in human use as a test antigen to determine the presence or absence of horse serum sensitivity of potential recipients of other injectable products derived from horse serum. Normal Horse Serum is provided as a test antigen with some of these products particularly the antivenins. The details of sensitivity testing procedures are specified in the labeling provided with the individual products. Both conjunctival and skin tests are described.

This test antigen is provided as normal unfractionated horse serum, whereas most horse serum therapeutic products are gamma globulin fractions of the native antibody-containing horse serum. Positive reactions are a common clinical experience in known horse serum sensitive patients. There is no evidence that normal horse serum is in any way inadequate as a test antigen for horse serum sensitivity of recipients, or that the use of the globulin fraction of normal horse serum would provide a better, more specific, more sensitive, or more reliable test antigen. Effectiveness has been demonstrated by the patients' responses to the subsequently administered horse serum products.

The product labeling does not contain warning statements with regard to the interpretation of conjunctival tests, particularly in children. Unless the person being tested by this method is watched continuously during the entire period of the test and irritation by rubbing of the eye is prevented, test results may be falsely interpreted as positive, particularly by inexperienced observers. This problem is most significant in children who tend to react to the instillation of anything into the eyes by rubbing them. This may delay or possibly prevent a therapeutic administration of a horse serum product. Also, severe conjunctivitis can be produced in hypersensitive people by the test. Informed opinion generally indicates that horse serum products should rarely if ever be injected without prior performance of a skin test. Thus the utility of the conjunctival test is low. At most, it may provide confirmation by a less sensitive means of the results of the skin testing procedure which provides a significantly better method of objective evaluation and control.

Recommendations. This material is safe, effective, and necessary for the sefe therapeutic use of horse serum products. Normal Horse Serum should be placed in Category I.

Labeling for horse serum products should call attention to the limitations of conjunctival tests if they are to be mentioned and should require performance of an appropriate skin test with Normal Horse Serum or the horse serum product itself prior to therapeutic administration of any horse serum based product, as noted in the discussions of the antivenins.

23. Blood Group Substances, A, B, and AB

Generic Statement

Review of Products Manufactured by Armour Pharmaceutical Co. and Pfizer, Inc.

1. *Introduction.* The blood group characteristics of red cells are inherited by Mendelian laws and are almost always constant throughout life. The ABO blood groups are defined by the presence or absence of antigens A and B or the red cell surface and the reciprocal presence or absence of two antibodies, anti-A and anti-B, which occur in the plasma when the corresponding antigen is mission. Blood group A and B antigenic substances are not confined to the red cell; they occur on the cell surfaces of probably all endothelial cells and of many epithelial cells. Substances capable of inhibiting either anti-A or anti-B activity are also found in water soluble form in body secretions. The ability to secrete or not secrete these materials is inherited in Mendelian fashion. Secretions such as saliva, gastric juice, seminal fluid, urine, and meconium, as well as pathological secretions, e.g., ovarian cyst fluid, contain A and/or B substance on the basis of this genetic mechanism. Horse and swine gastric secretions, and the mucosae from which they are derived, contain polysaccharide substances which are immunochemically almost identical to the A and B substances of human origin. Indeed, A and B-like substances are widely distributed in nature.

a. *Isolation.* These materials are usually extracted from pepsindigested horse or swine gastric material using cold 95-percent phenol followed by ethanol precipitation. Normally, several successive extraction-precipitation cycles are employed. Sometimes, further precipitation is accomplished with saturated ammonium sulfate at 60° C and even further purification can be performed using column chromatography.

b. *Chemistry.* The blood group substances derived in this way are macromolecules, classified as glycopeptides with molecular weights ranging from 2 X 10^5 to several million daltons. Approximately 85 percent of the weight of these molecules is carbohydrate. Both the A and B substances contain the five sugars, L-fucose, D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetylneuraminic acid.

c. *Immunological characteristics.* Because of their marked immunochemical similarity to human blood group substances, porcine blood group substance A can be used to stimulate anti-A production in blood group B or O individuals and equine blood group substance B can be used to stimulate anti-B antibodies in blood group A or O individuals. Equine blood group substance B preparations usually also contain measurable quantities of blood group substance A activity.

2. *Uses.* The present principal use of blood group substances A and B is for the in vivo stimulation of anti-A and/or anti-B antibodies in donors whose plasma is to be used for the preparation of diagnostic anti-A and/or anti-B reagents. Former uses of A and B substances were for specific neutralization of anti-A or anti-B antibodies in vitro for out-of-group blood transfusions, and for in vitro neutralization of unwanted anti-A or anti-B antibodies in the preparation of anti-Rh and other similar diagnostic reagents. These are no longer valid uses for the licensed biological blood group substance products.

When blood group substances A and/or B were used to neutralize A and/or B antibodies in Group O blood transfusions given to A, B, or AB recipients, 3-, 10-, or 25-mg doses were ordinarily employed. Between 1956 and the general discontinuation of this use in 1965 and last manufacture in 1969, approximately 100,000 3-mg doses were estimated to have been distributed by one firm for administration to recipients by this route without record of adverse effects (Ref. 5). Blood group substance products, not subject to licensure as biological drugs, are currently sold for use in anti-A or B neutralization steps in the in vitro processing of diagnostic reagents.

3. *Potency.* Licensed materials must have a potency sufficient to neutralize specifically anti-A or anti-B antibodies in vitro as outlined in the procedure in Ref. 8.

Although it was originally thought that differences between naturally occurring and hyperimmune anti-A or B antibodies could be discerned by their patterns of absorption or neutralization by soluble blood group substances, this no longer appears to be the case. Licensed blood group substance products must, however, satisfactorily meet arbitrary minimum potency test requirements. This test determines their immunologic reactivity on the basis of their capacity to inhibit the agglutination of group A red blood cells by anti-A or group B red blood cells by anti-B. In the future, test methods for licensed blood group substances may be developed wherein the products will be compared to reference standards which have been selected to meet the defined conditions.

4. *Safety and effectiveness.* Between 1967 and 1970, 117 donors received A, B, or AB substances for immune stimulation; 25 were successfully stimulated, of whom 17 were strong responders. There was no mention of any adverse reaction (Ref. 4).

In a review (Ref. 5) of 341 immunized subjects the following was observed:

One subject had slight fever with pain at the injection site; two of 219 subjects had mild systemic reactions following the injection; one subject had a nonfatal anaphylaxis.

In general, there were no significant reactions in subjects receiving 30 mg of A or B substance. The manufacturer estimated that a few thousand persons are deliberately immunized each year.

Until early 1977 when the Bureau of Biologics published immunization guidelines, two or three subcutaneous injections of 1- to 3-mg doses were often given at intervals ranging from 2 to 4 weeks (Ref. 7). Approximately 15 percent of the immunized subjects responded with insignificantly elevated isoagglutinin titers. Minor side effects, such as cutaneous wheal reactions, occurred in 1.8 to 3.6 percent of these subjects and were easily treated with an antihistamine.

TABLE 1.—ANTIBODY STIMULATION BY BLOOD GROUP SPECIFIC SUBSTANCES (REF. 5)

Site	Stimulus used	Route of admin.	Number of subjects	Percent satisfactory response	Percent minor side effects	Source of stimulating substance
Knickerbocker Laboratories	A	IM	<1,000	15	1.8-3.6	Pfizer.
Bio-Lab, Inc.	A, B	IM	673	58	3	Metric.
Plasma Derivatives	B	IM,SC	25	82	4	Other.

More recent data indicate that a relatively high proportion of subjects will make adequate immune responses if they are not immunized too frequently.

Armour Pharmaceutical Company in studies cited below (Ref. 4) required an eight-fold increase of anti-A or B titer above baseline or a response titer equal to or greater than 1/256 as a definition of an acceptable immune response.

In one study, Armour's objective was to elicit increased anti-A production

using 1 mg/mL intramuscular injections of blood group substance A into group B subjects. Of 89 recipients, 32 of whom were lost to followup, 63 percent had good responses. The rest yielded unacceptable titers even with a second injection given on the third to eleventh day. Of these subjects, 19 had been previously immunized (mean of 22 months earlier; range 6 to 32 months earlier). Thirty-eight subjects had had no prior immunization. Evaluation of subgroup responses within this study revealed the following:

a. Subjects with no prior immunization who were given one injection yielded 100-percent acceptable responses.

b. Subjects with no prior immunization who were given two injections yielded 88-percent acceptable responses.

c. Subjects with prior immunization who were given one more injection yielded 50-percent acceptable responses.

d. Subjects with prior immunization who were given two more injections yielded only 23-percent acceptable responses.

These data indicate that there was no improvement in responsiveness following two closely spaced injections of blood group substances and that the best responses occurred in subjects who had no prior immunization.

In a parallel study by the Armour Company designed to elicit increased anti-B production in group A subjects, a 0.9-mg intramuscular injection was preceded 2 to 21 days earlier by a 0.1-mg intradermal test injection. Of 97 recipients in this study, 29 of whom were lost to followup, 64 percent had acceptable responses.

The subgroup responses were as follows: In all instances the first does was 0.1 mg. When the second 0.9-mg dose was given 2 to 3 days later, 81 percent of the subjects had an acceptable response. If the second injection was given 4 days later, 52 percent had acceptable responses, and if it was given 5 to 21 days later, only 32 percent had an acceptable response.

These data suggest that waiting for more than 3 days between the skin test and the intended immunization caused the skin test dose to interfere with the response to intramuscular immunization.

In another pair of studies by Armour using A and B blood group substances for immunization of group O subjects, 1 mg of each substance was given in divided doses, both on the same day (0.1-mg intradermal plus 0.9-mg intramuscular) to a group of recipients with no prior immunization with these antigens. In 55 group O subjects, with 15 lost to followup, there were 75 percent who had good responses with anti-A. With anti-B, with 14 subjects lost to followup, there were 68 percent who had good responses. In a second similar study these responses in group O subjects were observed: With anti-A, of 51 subjects with 13 lost to followup, 78 percent had good responses; with anti-B, of 51 subjects with 14 lost to followup, 56 percent had good responses. In these studies, there were no adverse reactions noted except for occasional pain and swelling at the injection site.

From these studies, it appears that blood group substances A and B can elicit simultaneous anti-A and anti-B responses in group O subjects. Blood group substance A appears to be slightly more immunogenic than substance B. Both products are relatively safe.

Other data from Ortho Diagnostics, for a recently licensed product not subject to this review, further indicates the safety and effectiveness of these substances in stimulating anti-A and anti-B antibodies (Ref. 6).

Some data also exist about the use of A, B, or AB substance in vivo for neutralization of anti-A and B antibodies in factor VIII concentrate preparations. This is not an indication in the labeling of these products. Although significant hemolysis is recognized when large amounts of factor VIII materials (wet and/or dry) are administered, only anecdotal data are available about the effectiveness and safety of in vivo use of A and B substance to prevent such hemolysis. Preliminary observations have revealed that the substances can be mixed with factor VIII preparations in vitro given to reduce or eliminate in vivo hemolysis without observed adverse reactions (Ref. 7).

5. *Label analysis.* Pfizer, Inc., made no request to update labels, nor has it submitted any product lots for release for several years. Their production is, therefore, essentially defunct and their labeling is obsolete. Their labels have no clear information on actual dosage, side effects, precautions (such as asepsis), contraindications (regarding allergy in general or specifically regarding equine or porcine proteins), or warning regarding use in female subjects for in vivo stimulation.

The newly submitted labeling of Ortho Diagnostics, Inc., and Armour Pharmaceutical Co. is acceptable.

6. *Benefit/risk evaluation.* Side effects are so infrequent that despite varying rates of successful stimulation of anti-A or B antibodies, blood group specific substances for this purpose should continue.

7. *Recommendations.* a. The products should be placed in Category I on a generic basis. The product made by Armour should be placed in Category I. Pfizer's product should be placed in Category IIIB because of obsolete labeling and because no current effectiveness data are available.

b. Bureau of Biologics guidelines and manufacturer's labeling should indicate that reimmunization of subjects within less than a year is likely to result in little or no response.

c. Further studies for other uses are recommended. Although Cryoprecipitated Antihemophilic Factor redissolved in saline solution and antihemophilic factor concentrate can be administered, at least in moderate quantities, without regard to the blood group of the recipient, under some circumstances a hemolytic state is induced. This reaction can be managed by administration of group specific cryoprecipitated antihemophilic factor, but at times this is not practical. The use of A and B substance to neutralize the residual anti-A and/or anti-B content of pooled cryoprecipitated antihemophilic factor and antihemophilic factor concentrate should be investigated as an approach to this problem.

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24. Cobra Venom Products

Generic Statement

Cobra venom is obtained from mature cobra snakes (Naja naja). Lyophilized venom is subsequently fractionated, reduced to a powder, and later reconstituted as cobra venom solution. It was marketed by Hynson, Westcott &

Dunning, Inc., Baltimore, MD, as "Cobroxin"™ and in combination with Silicic and Formic Acids as "Nyloxin"™. The beneficial effects claimed from its parenteral administration is the alleviation of intractable pain.

Three main activities of cobra venom fractions are recognized: the hemolytic factor (phosphatidases); the proteolytic factor (proteases); and the neurotoxic factor (neurotoxins). The fractionation procedures for obtaining the cobra venom product were designed to eliminate the hemolytic and proteolytic factors but to retain the neurotoxic factor. Cobra venom solution is reported to have no effect on hepatic and renal function or blood clotting. It has no addictive properties and does not cause gastrointestinal dysfunction.

Most of the published reports on the use of cobra venom appeared during 1930 to 1945 (Refs. 1 through 33). The pain treated was associated mainly with malignant conditions or was secondary to various manifestations of arthritis. Cobra venom has also been suggested for the relief of pain associated with chronic angina, vascular crises, migraine headaches, and neuroses. Relief of pain in malignant diseases has been reported in as high as 60 to 70 percent of patients so treated. Cobra venom has been mentioned in a few subsequent reports (Refs. 34 through 46), but the Panel has found only one published report relative to human use in the last 10 years (Ref. 47).

Cobra venom solution was first marketed in 1952, but has not been advertised to the medical profession following 1957. Only a few thousand ampules were marketed each year.

1. *Evidence of effectiveness.* There have been no placebo-controlled or double-blind studies of pain relief with the cobra venom products; thus there is only anecdotal evidence of effectiveness. In the latest report (Ref. 47) only 5- to 10-percent effectiveness is claimed and then only after a trial period of 4 to 6 weeks.

2. *Evidence of safety.* Occasional severe vomiting or abdominal cramps have been reported (Refs. 21, 34 and 35). Other reactions observed most frequently are hives, diplopia, and dizziness. Cobra venom solution must be administered by deep intramuscular injection to prevent skin and subcutaneous reactions. The only contraindication to the use of cobra venom solution cited in the literature is its use in the presence of acute or chronic respiratory infection (Refs. 25 and 34).

3. *Benefit/risk evaluation.* There appears to be little substantiated benefit and no known serious risk. If any therapeutic effect from cobra venom solution can be demonstrated by appropriate studies then it may be of value in an attempt to reduce narcotic addiction in chronic pain.

4. *Recommendations.* It is recommended that Cobra Venom Solution and Cobra Venom with Silicic and Formic Acids (Cobroxin and Nyloxin) be placed in Category IIIB. During the Panel deliberations, the licenses were voluntarily withdrawn by the manufacturer on May 17, 1978 for Nyloxin and September 30, 1979 for Cobroxin.

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25. Human Plasma as a Source for Fractionation Products

1. *Plasma as a source for fractionation products.* Human plasma is the source material for albumin, plasma protein fraction, immunoglobulins, factor VIII concentrate, factor IX complex, and blood grouping reagents. The major clinically useful plasma fractions that were prepared when human blood was collected in glass bottles were albumin and gamma globulin (Ref. 1). Since these products are relatively stable on storage, it was possible to salvage plasma from whole blood stored at refrigerator temperatures for 21 days. Although the red cells are no longer suitable for use, the recovered plasma can be used as a source of derivatives such as albumin and gamma globulin.

When blood is collected in glass bottles, the container must be entered to remove the plasma following centrifugation of the bottle or sedimentation of the blood. Because of the potential danger of bacterial contamination, the remaining red cells must be used within 24 hours or discarded. The basic criteria for donor selection when collecting blood in glass bottles are the same as for whole blood to be used for transfusion purposes. The limiting factor for frequency of donation by this procedure is the donor's ability to regenerate red blood cells and maintain iron stores.

As methods were developed for preparation of the labile coagulation product, factor VIII (antihemophilic factor, AHF), it became necessary to freeze the plasma within a few hours after collection of blood from the donor (Refs. 2 and 3). Plasma salvaged from whole blood stored at refrigerator temperatures for more than 4 to 6 hours is not suitable as a starting material for preparation of factor VIII because of the attendant loss of activity (Ref. 4). If AHF concentrates are to be prepared, it is now the usual practice to use only plasma frozen while fresh and stored at −20° C or lower.

2. *Plasmapheresis.* Plasmapheresis is a procedure whereby plasma is removed from whole blood and the cells are returned to the donor as an autotransfusion. The technique had been known for many years (Ref. 5), but with the supplies and equipment available earlier it was a cumbersome and inefficient procedure to obtain plasma. With the availability of plastic transfusion equipment it became possible to separate plasma from cells in a closed system. Thus, with little danger of contamination, it was possible to separate the plasma from whole blood and return the cells to the donor (Ref. 6).

When plasmapheresis is carried out, the donor's ability to regenerate cells and to maintain iron stores are no longer limitations on the frequency of donation of plasma. With the technique of plasmapheresis, the limitations are the volume of blood that can be removed safety from the circulation acutely and the donor's subsequent ability to restore the protein which has been removed. The amount of blood that can be removed acutely depends on the donor's blood volume, which is proportional to body size. On the basis of considerable experience with collection of whole blood, it was established that at least 450 mL of blood can be removed without jeopardizing the health of the donor. This is now the standard transfusion unit. It became general practice in plasmapheresis to collect a unit of whole blood, separate the plasma, and return the cells to the donor and then collect a second unit of blood and repeat the process. In this manner, two units of about 250 mL each of plasma can be obtained at one time. The deficit in the donor's circulating blood volume is promptly restored by a shift of fluid from the interstitial space as well as by fluids administered during the blood collection and the autotransfusion of the cells. Since there are approximately 6 g/dL of plasma proteins, the collection of 500 mL of plasma represents a loss of approximately 30 g of protein with each such procedure.

3. *Donor safety considerations.* The frequency with which plasmapheresis donations can be made without jeopardizing the donor's health depends on the rate at which the lost proteins can be resynthesized. During intensive plasmapheresis over a short period of time in which 5 liters of plasma were removed in 5 days, normal donors replaced up to 49 grams of protein per day (Ref. 7). Protein loss in excess of this amount was reflected in a decrease in the total serum protein (Ref. 7). Two to 4 weeks were required for total serum protein and albumin to return to normal levels after this procedure, but 1 to 3 months were required for the gamma globulins to return to normal levels (Ref. 7).

Of the several different plasma proteins, albumin has been studied more extensively. Even for albumin, there are many unanswered questions regarding its homeostasis in health and disease (Ref. 8). It is apparent that the amount of albumin in the plasma represents only about half of the total body albumin pool. The remainder is in interstitial fluids. Following loss of plasma there is a shift of this albumin into the circulating plasma. The plasma protein level is promptly restored toward normal following the completion of a plasmapheresis procedure even though there is a net loss of protein (Ref. 6). On the basis of studies done in patients that lose plasma proteins over a period of time as a result of burns, renal disease or certain gastrointestinal disorders, it is apparent that there is a limit to the body's ability to compensate for protein loss (Ref. 9). The ability to compensate depends on a number of factors including the general health of the individual and his/her nutritional status (Ref. 9). Although the clinical manifestations of overt deficiency of albumin are well recognized, the possible effects of a borderline deficiency are as yet poorly defined. The procedure to determine total serum protein is relatively simple and rapid. The procedure to determine the amounts of specific plasma proteins is more complex. It is generally considered that, as long as the plasmapheresis donor is able to maintain total plasma proteins at established normal levels, the protein loss being incurred is not detrimental to the individual. However, there is no simple and direct way to determine the protein content of extravascular spaces so that protein reserves cannot be evaluated (Ref. 10).

There have been several retrospective studies in which accumulated data obtained from individuals participating in less intensive plasmapheresis programs have been evaluated (Refs. 11 through 13). Although these studies provide valuable information on the effects of long-term plasmapheresis on selected individuals, the primary objective was to obtain plasma and not to test the safety of the procedure. Because of the nature of the studies, it is inevitable that those who tolerate the procedure best will elect to continue, while those who do not tolerate the procedure may elect to withdraw from the program voluntarily. In those individuals who participate as plasmapheresis donors for only a short while, it is not clear whether they withdraw because of adverse changes in their health or for some other reason. However, the studies do indicate that as a selected group, plasmapheresis donors can maintain normal plasma protein levels while losing up to 1,000 mL of plasma, about 60 grams of protein, per week (Refs. 11 through 13).

It is of significance that even in a group of plasmapheresis donors selected because of obvious good health and robust nutritional status, changes in plasma proteins occurred (Ref. 13). During the first 4 months an increase in alpha globulins occurred, with a decrease in immunoglobulins. Thus the total protein concentration may remain constant since the decrease in immunoglobulins will be masked by the increase in alpha globulins. In individuals continuing on plasmapheresis for more than 6 months there was a trend toward levels, but the IgM level continued to decrease in those donors studied for up to 3 years.

These changes occurred in individuals that were able to maintain a total protein above 6 g/dL; and when, if there was a trend toward a significant decrease in the measured proteins, the intensity of the plasma withdrawal from these donors were decreased. Although the clinical significance of decreased immunoglobulins is not entirely clear, it is apparent that individuals participating in an intensive plasmapheresis program can become partially deficient in immunoglobulins. For the present it appears that there should be careful monitoring of each patient's immunoglobulins, particularly during the first 6 months, and if total protein, albumin, or immunoglobulin levels fall below the established normal range, the procedure should be discontinued at least until normal protein levels are reestablished.

In some studies it has been noted that enzymes such as serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) become elevated in individuals undergoing intensive plasmapheresis. These enzymes are associated with cellular activity of the liver and when present in the serum in increased amounts are considered to be an indication of hepatic dysfunction. It is not clear if this represents a physiologic response to increased demands for the synthesis of proteins or represents clinically significant impairment of hepatic function. It may be that a change in these enzymes might be an early indication of excessive protein loss. Currently available data are inconclusive on this point.

Other risks of plasmapheresis to the donor include the possibility that the wrong red cells will be returned to the donor. Thus the technique used must include a system for positive identification of the donor and donor's cells.

4. *Safety of plasma obtained by plasmapheresis.* As with all blood products there is a risk that the plasma obtained by plasmapheresis may be responsible for the transmission of hepatitis. Although the risk has been reduced by the requirement that all donors be tested for HBsAg, there is still a risk of both hepatitis B and so-called "non-A" and "non-B" hepatitis which is not detected by present tests. This risk is minimal in products that can be inactivated by heating such as albumin and plasma protein fraction. Products such as factor VIII concentrate and prothrombin complex concentrates which cannot be heated carry a major risk of transmitting hepatitis.

5. *Evaluation of plasmapheresis regulations—a. Medical review before repeated donations.* The protein loss resulting from removal of approximately 500 mL of plasma as currently allowed in a plasmapheresis procedure is equivalent to that occurring from the collection of a unit of whole blood. With whole blood donation there is a loss of protein in red cell hemoglobin in addition to the plasma proteins. However, the protein loss due to the donation of whole blood is not apt to be a problem because of the limitations on frequency of donation. Provided that plasmapheresis is done no more frequently than is being recommended by the Panel for donation of whole blood (four times per year) the protein loss would be of no greater consequence than that in whole blood donors. However, if plasmapheresis is done more frequently, protein abnormalities may result. To evaluate this possibility, specific plasma protein determinations must be done periodically in the more intensely plasmapheresed donors. It appears more appropriate for a qualified physician to review all the accumulated information periodically in order to evaluate whether or not an individual should continue in an intensive serial plasmapheresis program than to perform a physical examination prior to the initial plasmapheresis procedure.

b. *Total serum protein determinations.* Although there are differences of opinion regarding the clinical significance, there is adequate information to indicate that individuals undergoing plasmapheresis as allowed by the current regulations do experience changes in plasma proteins. The Panel believes that plasmapheresis should not be done unless the total serum protein determined prior to donation is within the established normal range.

c. *Specific plasma protein determinations.* A blood sample must now be obtained prior to the first plasmapheresis for determinations of specific plasma proteins. Currently, the results of such tests must be available and be found to be within normal limits within 21 days after the sample is drawn. This would allow collection of the plasma from 6,000 mL of blood prior to evaluating the specific plasma proteins. The results of these tests should be evaluated sooner to protect the donor that may have preexisting protein abnormalities.

d. *Physical examinations.* The panel considered on several occasions the requirement that each donor shall be examined by a qualified licensed physician on the day of the first donation or no more than 1 week before the first donation and at subsequent intervals of no longer than 1 year. It was noted that prior to the donation of whole blood and at subsequent plasmapheresis donations, the examination is permitted to be done by someone under the physician's direction. There are informed opinions that this requirement and the requirement for determination of specific plasma proteins will limit the participation of volunteer donors in plasmaphereses programs (Ref. 14).

e. *Donation frequency.* Current regulations allow the collection of 2,000 mL of whole blood per week. Thus it would be possible to retain approximately 52 liters of plasma per year. On the basis of anecdotal information, relatively few individuals are actually plasmapheresed for a long period of time or at the maximal rate. It appears that most individuals are plasmapheresed at the maximum