Appendixes
Appendix A- The Blood Resources Program, Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute

The National Heart, Lung, and Blood Institute through its Division of Blood Diseases and Resources (DBDR) supports research to improve the quality, safety and availability of blood and blood products for therapeutic uses. The DBDR in collaboration with members of the scientific community recently completed a planning study in which research needs and opportunities in the field of blood resources were delineated. A summary of the recommendations that emerged from this study are underscored below. Immediately following each recommendation are brief descriptions of related research projects currently supported by the DBDR.

**BLOOD BANK MANAGEMENT**

Better methods of collecting, separating, transferring, and preserving the cellular and liquid portions of blood are needed. These achievements depend upon developments in instrumentation, techniques, and automation.

*A platelet harvesting device is being developed to separate platelet-rich plasma from blood, based upon a centrifugal elutriation technique. This device should improve the amount of platelets recovered and reduce the contamination of platelet-rich plasma by erythrocytes and leukocytes.

(P43 HL-31873)

*Investigators are employing counter-current distribution techniques to separate cells and membranes in a two-polymer aqueous phase systems. This technique has been shown to be a sensitive, versatile method for characterizing and fractionating cell subpopulations based upon their surface or membrane properties.

(R01 HL-24374)

*Studies are being performed to test the feasibility of using pressure, generated by ultrasonic waves, to separate cellular elements of blood from plasma.

(R43 HL-31890)

*A regional blood distribution system using computer bar codes to identify all products, is being developed and tested at a large metropolitan blood center. Results indicate that a uniform supply of blood could be maintained at all hospitals while reducing regional outdated.

(P01 HL-09011)

1 Provided by George Nemo, Ph.D., Chief, Blood Resources Branch, DBDR, NHLBI
More effective data collection on blood and blood resource usage would help guide the development of predoctoral and postdoctoral education programs and provide a basis for exploring the need for autotransfusion, blood substitutes, and related issues of blood management.

In June, 1984, the NHLBI will initiate a study to determine future blood data collection, analysis, and reporting activities. The study will be conducted over a period of nine months. Technical support will be provided by a contractor experienced with medical records, medical abstracts, and standard coding and reporting systems. The study will involve a number of Federal agencies including the National Center for Health Statistics, the Food and Drug Administration, and the Department of Defense as well as major blood banking organizations.

CELLULAR ELEMENTS

An improved ability to reduce damage to the cellular elements from storage will depend on a better understanding of the factors responsible for the loss of viability and function of these cells. Basic research to understand the metabolic processes involved, the function of the cellular membrane in these activities, and how external forces interact with these features is critical to progress in this area.

Biochemical and ultrastructural studies are being performed to characterize the structure of the platelet cytoskeleton. These studies are expected to provide basic information relevant to the practical aspects of separating, storing and preserving platelets for transfusion.

(P01 HL-29583)

Studies of the relationship between surface saccharides and senescence in normal red blood cells have shown that the glycoconjugates of the cell membrane play a significant role in aging and sequestration of old erythrocytes.

(RO1 HL-17881)

A blood bag is being developed of semipermeable membrane material that will permit glycerolization and deglycerolization of blood cells without entry into the blood bag and thus insure sterility.

(RO1 HL-24466)

Studies are being performed to define the morphologic, metabolic, and functional changes that occur during storage of platelets in the liquid state. Information gained from these studies will be used to develop techniques to permit storage of platelets for one week or more with minimal loss of viability and function.

(RO1 HL-20818)

Baseline studies of changes in lipids and proteins of red cell membranes have demonstrated that improved methods of erythrocyte preservation result
in a significant reduction of lipid loss. These studies have also shown that red blood cells lose pieces of membranes in vivo, supporting the finding that the membranes of older cells contain less lipid than membranes of younger cells.

(R01 HL-25867)

Frozen preservation of blood cells, including bone marrow and stem cells, would expand the range of therapeutic modalities available to the clinician in many conditions for which there is limited therapy at present. In addition, culture techniques for growing bone marrow stem cells, and other cellular elements should be developed that permit the exploration of new therapeutic approaches.

Research is being performed on the cryopreservation of human platelets and granulocytes. Studies are focusing on the use of varying concentrations of cryoprotectants to achieve good recovery of functioning cells.

(R01 HL-27537)

Immunologic characteristics of the cellular elements have long been recognized as important aspects of their viability and function. Better methods to identify important immunologic features of cells and a better understanding of the clinical function of specific antigenic determinants will aid greatly in providing matched cells for therapeutic use. HLA registries, particularly for platelet and for bone marrow donors, are already required, and this need will undoubtedly extend to the other cellular elements as well.

Efforts are underway to determine the role of the HLA complex in the control of immune responses. Initial studies are focusing on the in vitro association of HLA phenotypes with cellular responses to artificial antigens.

(PC1 HL-09011)

A study of the membrane biochemistry of Rh antigens is providing information suggesting that the Rh antigen is associated with the main glycoprotein, band 3, of the red cell membrane.

(R01 HL-23108)

Investigators are developing and applying immunochemical methods to detect and characterize structural variations of HLA Class I and Class II cell-surface glycoproteins. Specific HLA gene products will be isolated and defined on the basis of molecular weight. Attempts will be made to relate the newly identified structural components to specific HLA-D region genes.

(PC1 HL-29583)

Investigators are determining molecular weights of Rh antigens, as well as other antigens in the red cell membrane. These studies have shown that the molecular weight of solubilized Rh antigen is over 500,000 daltons.

(R01 HL-24009)
Investigators are attempting to produce human monoclonal antiplatelet antibodies using spleen cells obtained from patients with immune platelet disorders. If monoclonal antibodies can be produced, useful reagents will be obtained that will enable investigators to identify antigen expression during the clinical course of immune platelet disorders.

(ROI HL-29513)

Using allospecific hybridoma antibodies, investigators have shown that spondylarthropathy is associated with the presence of HLA B27 antigens in the cells of patients. These data are significant because they demonstrate that the use of allospecific monoclonal antibodies may improve identification of susceptibility markers of inherited diseases.

(ROI HL-29572)

An International Bone Marrow Transplant Registry has been established for the purpose of collecting, analyzing and disseminating data on bone marrow transplantation performed at medical centers throughout the world. This registry is concerned with the identification of factors that affect graft and patient survival. These factors include pre-transplant transfusion, donor compatibility, granulocyte transfusion, and infection.

(Intra-agency Agreement with NIAID)

A large number of substances are actively transported on, or secreted by, cellular elements. Some are recognized to be of significant physiological importance. The myriad of potentially useful agents found in association with these cells, including the lymphokines, mediators of several varieties, enzymes, and other biologically active chemicals, must be isolated, identified, and studied. When important functions are identified, isolation and purification using monoclonal antibodies and other techniques.

A research project is focusing on the physiological functions of platelet-derived factors in the regulation of cellular growth, migration, and metabolism of specific target cells in culture. This project involves studies of platelet-derived growth factor, platelet factor 4, beta-thromboglobulin, serotonin and thrombin.

(PO1 HL-29583)

In an ongoing project, platelet-activating factor (PAF), a low molecular weight substance released by white blood cells, is being studied along with an inhibitor of PAF. Studies are focusing on the role of these molecules in several diseases, notably asthma and immunologically mediated lung diseases.

(ROI HL-25220)
PLASMA AND PLASMA DERIVATIVES

Perhaps the greatest immediate challenge in the area of plasma derivatives is related to the development of techniques that utilize existing or new methods of separation to isolate, purify, and prepare safe products for research and therapeutic use. Such advances in technology will not only lead to better, more abundant, and less costly products, but will also provide opportunities to isolate and study trace agents of the plasma that have important functions in relation to coagulation, inflammation, the complement system, and other important response mechanisms of the body.

In an ongoing study, arginal peptides are being synthesized for use in the affinity chromatographic purification of serine proteases. Arginal peptides attached to agarose resins will serve as a general affinity chromatography procedure in plasma protease purification, and the procedure may have therapeutic importance in the isolation of proteases for the treatment of hemophilia and thrombolytic disorders.

Work is continuing on the combined use of polyethylene glycol precipitation and Cibacron Blue Sepharose in the purification of several plasma proteins.

A study is being conducted to determine the feasibility of purifying alpha-1-antitrypsin from the plasma of normal donors for supplementation in infants with alpha-1-antitrypsin deficiency who have early symptoms of liver disease.

Specifically needed in this area (plasma and plasma derivatives) is the development of disease-free products, particularly reagents free of hepatitis virus, that can be used safely in clinical situations. There is also a need to identify the etiology of other adverse reactions caused by transfusion of plasma derivatives, such as those that occur with some clotting fractions and immune globulins.

A variety of chemical and physical inactivation methods are being employed to reduce the infectivity of non-A non-B hepatitis viruses (NANB) in labile blood derivatives. The efficacy of the inactivation process is being evaluated by inoculating treated material into chimpanzees.

Research is continuing on the development of new plasma derivatives which have existing or potential clinical use. A factor IX concentrate preparation has been developed that is non-thrombogenic. Methods are also being developed to reduce viral infectivity in factor VIII concentrates and similar methods are being applied to the production of factor X and Protein C concentrates.
SAFETY

With the virtual elimination of posttransfusion hepatitis caused by hepatitis B virus, more attention must be focused on identifying, isolating, and developing suitable antibodies and vaccines against non-A, non-B virus (NANB), which is now the prime cause of this disease. Efforts to minimize the occurrence of cytomegalovirus (CMV) and other less common agents as a cause of hepatitis, particularly in certain specialized patient populations, must be continued.

Investigators are attempting to identify and to characterize NANB hepatitis agents or antigens in the sera, liver extracts and tissues of infected humans and chimpanzees. If successful, new tests for NANB agents would be developed; immunization with purified NANB antigens would be evaluated in chimpanzees; in vitro cultivation of the agents would be attempted; and the epidemiology and natural history of NAHB hepatitis would be studied.

(PO1-HL-09011-18A1)

Attempts are being made to modify hepatitis B surface antigen (HBsAg) by chemical means in order to induce or amplify an immune response in nonresponder mice. If successful, these studies will be extended to humans and include HBsAg carriers and vaccinated individuals. In addition, interferon, which decreases the concentration of Dane particles and HBsAg in human carriers, will be encapsulated in polysomes or covalently linked to polysaccharides in an effort to direct the lymphokine to liver tissue. A separate project entails introduction of the HBV genome into eukaryotic cells in order to study viral expression.

(PO1 HL-09011)

A computerized serum and data bank, established in 1969, is projected to contain approximately 300,000 samples by 1985. Long-term surveillance studies continue to be performed with these serum samples to explore epidemiological patterns of NANB hepatitis.

(PO1 HL-09011)

Studies are focusing on the relationship of HLA-linked genes to immune responses that include vaccination with HBsAg or natural infection with HBV.

(PO1 HL-09011)

A repository of coded, frozen serum samples from the Transfusion-Transmitted Viruses Study is being maintained. These samples are available to investigators for hepatitis research, with approval of the National Heart, Lung, and Blood Institute.

(N01 W-27000)
A breeding colony of chimpanzee, presently consisting of 43 animals, is being maintained for hepatitis research.

(NO1 HB-27004)

A prospective investigation is being conducted to identify the agent(s) of NANB hepatitis. A rapid screening procedure, initially developed to detect anti-HBsAg, is being applied to the detection of the agent(s) of NANB hepatitis. Serum samples from NANB patients and liver homogenates from infected chimpanzees are being evaluated with this method.

(NO1 HB-37010)

A blood donor survey and serum sample analysis is being performed to correlate clinical manifestations of hepatitis with concentrations of liver enzymes in the blood. Studies also include the development of methods for detecting and isolating DNA in serum with elevated concentrations of alanine aminotransferase (ALT), and of methods for molecular cloning of the nucleic acid isolates.

(NO1 HB-37011)

The transmission of Epstein-Barr virus (EBV) by blood transfusion is being studied. Investigations are concerned with the frequency of EBV transmission, the frequency of clinical illness in infected individuals, antibody responses to parenterally derived infections, and the role of passive immunity and transfusion volume in the development of infection.

(R01 HL-30311)

A prospective study is being performed to determine the incidence of transfusion-associated cytomegalovirus (CMV) infections, including primary, reinfection, and reactivated latent infection in immunocompetent and immunodeficient blood recipients. Investigators will determine the clinical significance of CMV infection in these patients and will attempt to develop tests for determining which blood donors are capable of transmitting CMV.

(R01 HL-30329)

A longitudinal study is being performed to determine the prevalence of immunologic abnormalities in patients with hemophilia. These abnormalities may be induced by factor VIII treatment and are similar to the severe dysfunction of the immune system observed in patients with acquired immunodeficiency syndrome (AIDS).

(R01 HL-31015)

A study is underway to investigate the possible causative and contributing factors and their interactions in the pathogenesis of AIDS. A large population of healthy, but at-risk, homosexual men will be followed prospectively, using a variety of serologic and immunologic markers to define the sequence of events leading from good health, to altered immunity, to AIDS.

(PO1 HL-09011)
A study has been initiated to demonstrate whether an AIDS-inducing infectious agent is present in the plasma of patients with this syndrome. Plasma samples have been inoculated into chimpanzees that are being monitored for a variety of immune functions as well as clinical manifestations of the disease.

(YO2-HB-30006)

Investigators are attempting to determine whether patients with hemophilia A display immune function changes similar to those observed in patients with AIDS. In addition to hemophilia patients, the immunological status of patients with sickle cell anemia and thalassemia major will be evaluated, since both groups repeatedly receive substantial quantities of cellular blood products.

(YO1-HB-30034)

An outbreak of an acquired immunodeficiency disorder, which resembles human AIDS, has been observed in rhesus monkeys at the University of California Davis Primate Center. The disorder is referred to as Simian Acquired Immunodeficiency Syndrome or SAIDS. Recently, it was found that SAIDS could be experimentally transmitted to monkeys. A project is currently in progress to determine the role of blood and blood components in the transmission of this disease in monkeys.

(Y02-HB-30018)

Methods are being developed to detect circulating antigen-antibody complexes containing the putative agent of AIDS. A variety of laboratory procedures will be used to detect and characterize specific antigens and antibodies found in the sera of patients with AIDS.

(R01-HL-32434)

A study is being performed to quantitate specific breakdown products of nucleic acid metabolism including purine and pyrimidine bases, in the serum and urine of several study groups including patients with AIDS. It is hoped that this study will lead to the development of a laboratory test to detect asymptomatic carriers of AIDS.

(R01-HL-32432)

A study is being performed to determine the functional role and significance of human CMV, human T-cell leukemia virus (HTLV), and other agents associated with AIDS. Several experimental approaches, including transmission experiments in non-human primates, will be tried.

(R01-HL-32505)

A research program is underway to assess the value of a battery of assays to detect the carrier state of AIDS. These include tests for T-cell subsets using 10 diverse monoclonal antibodies, measurement of antibodies to the
three known types of HTLV, and measurement of immune complexes. Study groups will be followed prospectively for a three year period.

(ROI HL-32453)

A number of tests are being conducted that include serological assays (thymosin, microglobulin, alpha interferon, anti-HTLV, anti-HB core antigen and anti-CMV) and cell marker assays (helper T cells, suppressor T cells, B cells, natural killer cells, monocytes, DR-antigen-positive cells, and surface immunoglobulin positive cells) in an attempt to discriminate between healthy individuals and those who are asymptomatic carriers of AIDS.

(ROI HL-32477)

Methods are being developed that would make biological assays of human alpha interferon feasible for mass screening procedures. Studies will specifically focus on the ability of these assays to detect asymptomatic carriers of AIDS.

(ROI HL-32473)

DNA hybridization procedures are being applied to the detection of viral nucleic acid in lymphocytes of patients with AIDS, AIDS-related complex, homosexuals with immune abnormalities, and controls. Viruses to be studied include CMV, EBV, and HTLV.

(ROI HL-32471)

Donor safety is only now being viewed with any degree of interest. Because of the proliferation of apheresis techniques and the increasing use of single donors to provide large quantities of a reagent, more information must be developed on the threat that accompanies the loss of cellular or plasma constituents and the hazards posed by the repeated introduction of steroids and colloids into the circulation of the donor.

A project concerned with the effects of cytapheresis on the lymphoid system of donors is underway. This study deals with the short and long-term effects of repeated cytapheresis, particularly on the number, distribution, and function of lymphocyte subpopulations.

(ROI HL-09011)

APHERESIS

The technique of apheresis is being applied to many diverse clinical conditions. Research in the immediate future must deal with the development of new instrumentation, new immunoabsorbents, hazards to the donor, the clinical efficacy of newly emerging treatment strategies, and cost-benefit ratios of its large-scale use.
In a study of methods to reverse platelet alloimmunization, therapeutic apheresis and immunoabsorbent columns are being used to remove platelet alloantibodies.

(IMMUNOLOGY)

Although immunologic investigation is important in many of the topics already mentioned, new concepts are being associated with transfusions and specific blood cell antigens in organ transplantation. In addition, further work is needed on the use of extracorporeal systems to treat blood and bone marrow with monoclonal antibody.

(BLOOD SUBSTITUTES)

The clinical evaluation of existing and newly formulated perfluorochemicals represents an immediate challenge for the blood transfusion specialist. Although additional perfluorochemical reagents are being developed and new surfactants are being devised, research with other oxygen-carrying solutions should be pursued.

In an ongoing study, investigators are testing hemosomes, which are stroma-free hemoglobin solutions encapsulated in artificial phospholipid membranes, as red cell substitutes. Sterile hemosomes are being produced in sufficient quantities for safety and efficacy tests in laboratory animals.

New red cell substitutes, utilizing hemoglobin bound to polymeric compounds, are being synthesized and tested for safety and efficacy in laboratory animals. The polymeric compounds such as hydroxyethyl starch or dextran are coupled to hemoglobin to increase the circulatory dwell time of the oxygen-carrier in the circulation.

Investigators are determining oxidation-reduction equilibria of hemoglobin covalently modified with various organic phosphates. These studies will provide new information on modified hemoglobins which are of potential importance as red cell substitutes.

Investigators are exploring the use of tetrameric and polymerized hemoglobin solutions and perfluorochemical red cell substitutes in the treatment of moderate and severe anemia.

A perfluorochemical emulsion is being used to obtain hemoglobin-free rat neural tissue preparations that will permit characterization of cerebral
Intramitochondrial respiratory chain function in situ. The long-term objective of this project is to evaluate the potential therapeutic effectiveness of perfluorochemicals to prevent or reduce central nervous system metabolic damage caused by cerebrovascular pathology.

(RO1 HL 30100)

Investigators are using perfluorochemical emulsions of defined particle size as models of platelets and chylomicra in order to determine their distribution near the wall of microcirculatory vessels. This work combines methods of rheology, transport phenomena, and circulatory physiology. Its aim is to obtain a comprehensive rheologic picture of blood cell effects in flowing blood.

(RO1 HL 30087)

Investigators are studying the nuclear magnetic resonance (NMR) spectral characteristics of new and promising perfluorochemicals. It is hypothesized that perfluorochemical emulsions can accelerate the restoration of myocardial function following coronary flow reduction and that imaging could be used to monitor this process.

(RO1 HL 30104)

The efficacy of a number of selected perfluorochemicals in the in vitro perfusion of mammalian testis is being explored to determine the effect of these artificial oxygen carriers on testosterone secretion.

(RO1 HL 30083)

Clinical Trials

Throughout the entire range of subjects in the blood resources area, the need for controlled, statistically significant clinical trials repeatedly surfaces. Clinical indications for the use of the various blood fractions must be better delineated, and the appropriate use of the resource must be ascertained. Timely development of indications for the use of a product will not only assure the rapid application of techniques but also limit the use of costly, ineffective treatment modalities popularized by anecdotal reports and inadequate trials.

A multi-institutional clinical study is underway to evaluate the capacity of intravenously administered CMV immune globulin to protect high risk prelature infants against CMV infection acquired by blood transfusion.

(RO1 HL-29883)

Education

The authors of this report make frequent reference to the need to develop appropriate educational opportunities for users of blood fractions.
In this summary, the importance of this plea to provide proper training for those who administer blood is reemphasized, since both the success of clinical care and the control of health care costs are intimately related to the appropriate use of this vital resource. In addition, the factors that motivate or inhibit blood donors should be investigated, inasmuch as an understanding and sympathetic public is necessary for an adequate supply of blood.

In September, 1984, the NHLBI and the FDA will cosponsor an NIH Consensus Development Conference entitled, “Fresh Frozen Plasma: Indications and Risks.”

In 1983, the NHLBI implemented the Transfusion Medicine Academic Award. This program provides for the integration of educational programs in transfusion medicine into the medical school curriculum. The program will be announced each year until needs in transfusion medicine are fulfilled. “Transfusion Medicine” is defined as a multidisciplinary area concerned with the proper use or removal of blood and its components in the treatment or prevention of disease states (other than in renal hemodialysis).