Tenth Report of the Director
National Heart, Lung, and Blood Institute

Volume 4.
Blood Diseases and Resources

U.S. Department of Health
and Human Services
Public Health Service
National Institutes of Health

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Tenth Report of the Director
National Heart, Lung, and Blood Institute
Ten-Year Review and Five-Year Plan

Volume 4.
Blood Diseases and Resources

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Preface

The National Heart, Lung, and Blood Institute is now in its fourth decade, following its original establishment in 1948 as the National Heart Institute (P.L. 80-655). With a growing awareness of national health problems over the years, such as those reflected in the President's Conference on Heart Disease and Cancer (April 21, 1961) and the President's Commission on Heart Disease, Cancer, and Stroke (December 9, 1964), it was redesignated by the Secretary of Health, Education, and Welfare (now Health and Human Services) as the National Heart and Lung Institute (NHLI) in 1969. The activities of the Institute were expanded in 1972 by the National Heart, Blood Vessel, Lung, and Blood Act (P.L. 92-423) to advance the national attack on diseases of the heart, blood vessels, lungs, and blood. With the passage of the Health Research and Health Services Amendment in 1976 (P.L. 94-278), in which the NHLI was redesignated as the National Heart, Lung, and Blood Institute (NHLBI), the authority was further enlarged to include research on the use of blood and blood products and on the management of blood resources.

The 1972 act was of special significance. The law mandated that the Director of the Institute, with the advice of its Advisory Council, develop a national plan for attacking heart, blood vessel, lung, and blood diseases. The need for the plan evolved from a recognition that isolated approaches were no longer appropriate to the growing magnitude of these public health problems. Twenty-eight task groups of approximately 250 medical and scientific advisors assessed the understanding of these problems and identified new opportunities for initiatives. The effort culminated in the five-volume National Heart, Blood Vessel, Lung, and Blood Program (DHEW Publ. Nos. (NIH) 73-515, 73-516, 73-517, 73-518, 73-519, 73-520, 73-521, 73-522, and 73-524). The needs, goals, recommendations, and strategies presented in the document provided a National Program, which for the past decade has been updated annually and has guided the Institute. The process includes:

- Research on the epidemiology, etiology, and prevention of heart, blood vessel, lung, and blood diseases

- Research on basic cardiovascular biological processes

- Development and evaluation of techniques, drugs, and devices to aid diagnosis and treatment
- Programs to develop technological devices to assist, replace, or monitor vital organs
- Field studies and large-scale tests relating to those diseases
- Research on blood diseases and the use of blood resources in the United States, including such items as collection, preservation, fractionation, and distribution
- Education and training of scientists and clinicians
- Public and professional education programs in all aspects of those diseases
- Programs to research and study heart, lung, blood vessel and blood diseases of children.

The 1972 act also requires the Director of the Institute to submit an annual report to the President, for transmittal to Congress, on the accomplishments of the National Program during the preceding year and on plans for the next 5 years.

This five-volume Tenth Report of the Director, NHLBI, which is a 10-year review and 5-year plan, commemorates the 10th anniversary of the National Program. This volume reports on program areas of the Division of Blood Diseases and Blood Resources. It begins with an executive summary and a description of the magnitude of the problem. Progress, achievements, and future goals are then reported in the following areas:

- Thrombosis and hemostasis
- Red blood cells and their disorders
- Blood resources.

The volume concludes with discussions of prevention and of research training and development.

Volume 1 serves as an executive summary of the other four volumes. Volume 2 reports on program areas of the Division of Heart and Vascular Diseases, and volume 3 reports on programs of the Division of Lung Diseases. The final volume contains a discussion of important companion issues, including program coordination and liaison.

The process by which these volumes were developed was modeled after the one used in 1972 for the National Program. Members of working and review groups were drawn from the NHLBI staff, the National Advisory Council and advisory committees, the extramural scientific community, the community of health providers and health consumers, and the general public. Persons who participated in the development of this volume are listed on pages xiii to xviii.

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1. Executive Summary

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1. Executive Summary

With the reorganization of the National Heart and Lung Institute approved by the Secretary of Health, Education, and Welfare in July 1972, the Division of Blood Diseases and Resources (DBDR) was created. In the same year, three laws were enacted that mandated new activities and expanded the existing responsibilities of the NHLI in blood diseases and resources:

May 16, 1972 -- The National Sickle Cell Anemia Control Act (P.L. 92-294)

August 29, 1972 -- The National Cooley's Anemia Control Act (P.L. 92-414)

September 19, 1972 -- The National Heart, Blood Vessel, Lung, and Blood Act (P.L. 92-423).

In response to the National Heart, Blood Vessel, Lung, and Blood Act, a National Program was developed by the NHLI and transmitted to the Congress.

As a result of this process, five National Program areas were defined within the Division of Blood Diseases and Resources:

- Thrombosis and hemostasis (bleeding and clotting disorders)
- Sickle cell disease
- Red blood cell disorders
- Blood resources
- Biomaterials.*

Revisions of the National Heart, Blood Vessel, Lung, and Blood Diseases Act in 1976 (P.L. 94-278) changed the NHLI to the National Heart, Lung, and Blood Institute (NHLBI) and expanded the Institute's responsibilities for research concerning blood resources.

*The biomaterials program was transferred to the Division of Heart and Vascular Diseases in 1975.

In the decade since the passage of the National Heart, Blood Vessel, Lung, and Blood Act of 1972, the Division of Blood Diseases and Resources has expended $577.5 million to support research, research training, and prevention, education, and control programs. The amounts and percentages of funds for the National Program areas are given in table 1. The achievements resulting from these expenditures are outlined in this summary and are discussed in detail in the report itself.*

Table 1. Division of Blood Diseases and Resources
Cumulative Expenditures, 1972 to 1981

National Program Areas	Dollars in Millions	Percent
Thrombosis and hemostasis	$209.8	36
Red blood cell disorders	75.0	13
Sickle cell disease	189.5	33
Blood resources	99.0	17
Biomaterials	4.3	1
Total expenditures	$577.5	100

Magnitude of the Problem

For decades, the illnesses, deaths, and health care costs resulting from blood disorders were incompletely reported, and the quantities and costs of blood collected, processed, and transfused were not reported at all.

Recent research findings have revealed the widespread involvement of thrombosis in the pathology of many disorders; more aggressive therapy for cancer has resulted in increased susceptibility of patients to bleeding disorders; and many blood disorders either cause, accompany, or result from various types of diseases and injuries.

*The method by which this report was developed can be found on pages 42-44.

Recent studies have provided data with which accurate estimates can be made, for the first time, about the quantities of blood collected, processed, and transfused and about the numbers and types of patients who receive transfusions of whole blood and red blood cells.

Blood Diseases

Blood diseases are chronic disorders.* The genetic blood diseases, such as hemophilia, sickle cell anemia, Cooley's anemia, and G-6-PD deficiency, have lifelong effects on several million Americans. In the aggregate, they are the primary cause of death in as many as a thousand patients per year. Their chronic effects, however, range from mild to life-threatening and from transitory to continuous, and they result in major morbidity and cost for the health care system. The acquired diseases in which abnormalities of the blood are prominent, such as coronary and cerebral thrombosis, phlebitis, and other thromboembolic conditions, affect additional millions of Americans.

The following data illustrate the magnitude of the problem of blood diseases:

- In 1978, blood diseases were reported on death certificates as contributing factors in 579,846 deaths.
- In 1978, blood diseases were reported as the primary (underlying) cause of 351,322 deaths.
- In 1978, blood diseases contributed to 3,274,000 incidences of hospitalization.
- In 1978, blood diseases were the primary reason for the hospital stay in 1,378,000 of these hospitalization incidences.
- In these 1,378,000 hospitalization incidences, 16,130,000 days of hospital care resulted.
- In 1979, blood diseases were the reason for 6,695,000 patient visits to physicians' offices.

*Blood diseases here refers both to the conditions traditionally considered blood disease and to some patients with such diseases as myocardial infarction ("heart attack"), stroke, and pulmonary embolism, in which blood clotting plays a crucial role.

- In 1979, estimated direct costs for blood diseases (expenditures for hospital care, professional services, nursing home care, and drugs and medical sundries) were $7,689,000,000.

- In 1979, estimated indirect costs (costs of productivity lost because of illness and death) were $19,021,000,000.

The number of deaths (351,322) caused by blood diseases in 1978 is almost six times greater than the number previously presented in reports by the NHLBI. The larger number results from the inclusion of deaths for groups of diseases not previously reported as blood disorders. Their inclusion now is related to recent research showing that thrombosis is important in the pathology of at least two-thirds of acute myocardial infarctions and four-fifths of cerebrovascular diseases.

The diseases that caused the 351,322 deaths in 1978 caused 402,175 deaths in 1970. There was an overall decline of 13 percent during the last decade. Exclusive of deaths caused by acute myocardial infarction, which are discussed in NHLBI reports addressing heart and vascular diseases, deaths from blood diseases declined:

- 21 percent in persons from birth through age 14
- 21 percent in persons of ages 15 through 64
- 6 percent in persons 65 years of age and older.

In brief, fewer persons die from blood diseases today than a decade ago, and the rate of decrease has been greatest in persons under the age of 65.

Currently, blood diseases are only rarely preventable. Improved diagnosis and treatment, however, have resulted in fewer deaths. As might be expected, an increasing population of longer-living patients with chronic disorders requires more health care. Data about hospitalizations of patients with blood diseases confirm this expectation. The changes that occurred from 1970 through 1978 are:

- Total hospital stays to which blood diseases contributed increased by 40 percent (from 2.3 million to 3.3 million).

- Hospital stays for which blood diseases were the primary cause increased by 28 percent (from 1.1 million to 1.4 million).

- Days of hospitalization for blood diseases increased by 15 percent (from 14 million to 16 million).

In contrast, the average length of hospital stay per patient decreased by 11 percent (from 13.1 to 11.7 days).

Blood Resources

There are nearly two dozen blood products transfused to patients. These products range from whole blood to individual proteins separated from blood plasma. In 1979, the number of Americans from whom whole blood or a blood component was withdrawn was about 6 million. About 5.5 million donated whole blood, more than 250,000 gave plasma, and an estimated 50,000 donated white cells or platelets.

In 1979, hospitals and blood centers in the United States collected 10.8 million units of whole blood and used them to prepare:

3,550,000 units of whole blood for transfusion

7,250,000 units of red cells for transfusion

3,050,000 units of platelets for transfusion

2,050,000 units of plasma for transfusion

500,000 units of cryoprecipitated antihemophilic factor for transfusion

1,350,000 liters of plasma recovered for use by the pharmaceutics industry in the manufacture of plasma products.

The pharmaceutics industry purchased and collected more than 4 million liters of plasma in 1979 and prepared the following quantities of two blood products for U.S. consumption:

5,800,000 units of albumin and plasma protein fraction (12.5-gram units)

412,500,000 units of antihemophilic factor.

In 1979, 13.4 million units of five blood products were transfused, as follows:

2,200,000 units of whole blood

7,300,000 units of red cells

2,200,000 units of platelets

1,300,000 units of plasma

400,000 units of cryoprecipitated antihemophilic factor.

In addition, an estimated 5 million to 6 million units of albumin and plasma protein fraction and more than 400 million units of antihemophilic factor were transfused.

In 1979, 2.9 million patients received whole blood or red cells, or both, as a part of their therapy, as follows:

Cancer	More than 500,000
Cardiovascular and cerebrovascular disease	About 400,000
Gastrointestinal disorders	About 400,000
Trauma	About 350,000
Blood disorders, obstetrical problems, bone and joint disorders, and gynecologic and breast diseases	Between 100,000 and 200,000 each
Respiratory, liver, kidney, and bladder disorders	Between 50,000 and 100,000 each
Benign tumors, gallbladder, genital, metabolic, skin and soft tissue disorders, and complications of other disorders	Between 25,000 and 50,000 each
Infections, hernias, diabetic complications, pancreatic disorders, and nervous system disorders	Between 10,000 and 25,000 each

The principal adverse effect of transfusion of every blood product except albumin is transfusion-transmitted hepatitis. In 1979, about 250,000 recipients of blood resources were infected by a hepatitis virus. Most of these cases (85 to 90 percent) are type non-A,non-B hepatitis. Although most cases of non-A,non-B hepatitis produce no immediate debilitating symptoms, hepatitis caused by transfusion is still a major public health problem because a significant number of patients who contract non-A,non-B hepatitis eventually develop chronic liver damage.

Thrombosis and Hemostasis

The processes that maintain the fluidity of blood within blood vessels are known collectively as hemostasis. Normal hemostasis is essential for survival in species such as man, whose blood is pumped to the tissues under high pressure. Moreover, the hemostatic reactions have a major function in other of the body's defense mechanisms including the inflammatory response, wound healing, and the cellular immune response whereby the body rejects foreign tissue. Hemostasis is one of the key biologic processes that makes advanced forms of life possible.

Bleeding due to impaired hemostasis can result from an abnormality affecting the platelets, the blood coagulation reactions, the fibrinolytic reactions, or a combination thereof. A group of primary, hereditary bleeding disorders exists, of which hemophilia is the most important. Although there are only 10,000 to 25,000 persons with hemophilia in the United States, hemophilia is a major national health problem. The disease requires costly, lifetime treatment, and the preparation of enough factor VIII for the treatment of hemophilia causes a steadily growing demand on the nation's blood resources.

Much more common are the acquired bleeding states that are not diseases in themselves but are serious, sometimes fatal, complications of a wide variety of primary diseases in which one or more steps of the hemostatic process may break down. The disorders in which abnormal bleeding adds substantially to morbidity and mortality include: chronic liver diseases, chronic kidney diseases, disorders of the small intestine, complications of pregnancy and delivery, the leukemias, metastatic carcinoma, aplastic anemia, certain types of drug reactions, and autoimmune states, such as systemic lupus erythematosus, in which antibodies to platelets or blood coagulation proteins are formed.

Thrombosis, which is the formation of a blood clot within the lumen of a blood vessel, results from normal hemostatic reactions occurring under abnormal circumstances. Thrombosis occurs in arteries, in veins, and in the small vessels of the microcirculation of the tissues. A thrombus blocks or partially blocks the lumen of a blood vessel, with resultant cessation or impairment of blood flow to or from an organ or tissue. An embolus is material that breaks from a thrombus and occludes a vessel downstream.

In the United States, thrombosis and embolism are today's leading medical causes of death. Although thrombosis and embolism are not listed as diseases, they are pathologic events that serve as the cause of death in a number of primary diseases. The formation of a thrombus in an atherosclerotic coronary artery usually precipitates the acute myocardial infarction that kills the

patient. Similarly, a thrombus forming in a cerebral vessel is a frequent cause of fatal strokes. Venous thromboembolism is an ubiquitous and frequently lethal complication of a broad spectrum of medical and surgical illnesses. Hundreds of thousands of Americans are affected each year. Pulmonary embolism secondary to thrombosis in the deep veins of the legs is responsible for or has contributed to thousands of deaths annually in the United States.

Major Advances of the Past 10 Years

The advances in understanding normal hemostasis, bleeding disorders, and thrombosis over the past 10 years have been extensive and important. They derive in very large part from the efforts of biomedical investigators who have received research training support, research grant support, or both, from the NIH, and they include the following:

- Elucidation of the functions of prostaglandins, of platelet surface membrane glycoproteins, of von Willebrand factor, and of the platelet contractile mechanism in platelet adhesion and platelet aggregation.

- Purification to homogeneity of all the human plasma coagulation proteins except coagulant factor VIII; determination of the complete primary structure of fibrinogen and prothrombin; and delineation of the sites of limited proteolysis and resultant molecular changes associated with activation of the great majority of the coagulation proteins.

- Discovery of the role of vitamin K in the gamma carboxylation of glutamic acid residues of the vitamin K-dependent coagulation proteins; discovery of two new vitamin K-dependent proteins, protein C and protein S; and identification of protein C as a regulator of both blood coagulation and fibrinolysis.

- Elucidation of the tissue factor pathway for initiating blood coagulation through activation of factors IX and X; detailed characterization of the contact activation reactions for initiating blood coagulation; identification of the fragments of prothrombin formed during activation; and elucidation of the importance of reactions at the platelet surface for the activation of prothrombin during hemostasis.

- Elucidation of major functions of endothelial cells in hemostasis: synthesis of prostacyclin, vascular plasminogen activator, and von Willebrand factor; binding of

thrombin with resultant alteration of its substrate specificity and rate of inactivation.

- Determination of the primary structure of antithrombin III, its binding sites for heparin, and the kinetics of its interaction with serine protease coagulation enzymes in the presence and absence of heparin; delineation of properties of alpha-2-macroglobulin and mechanisms by which it binds and neutralizes proteases.

- Determination of the primary structure of plasminogen; identification of the lysine bindings sites of plasminogen and their function in fibrinolysis; identification of vascular plasminogen activator as the major plasma inhibitor of fibrinolysis.

- Discovery of the platelet mitogenic factor and of its role in the proliferative response of the vessel wall to injury.

- Identification of the platelet surface membrane abnormalities responsible for the hemostatic defects in Glazmann's thrombasthenia and in the Bernard-Soulier syndrome.

- Discovery of the effectiveness of plasma therapy in thrombotic thrombocytopenic purpura.

- Introduction of use of platelet concentrates into the general practice of medicine, particularly in relation to the treatment of cancer and thrombocytopenic conditions.

- Development of improved techniques for the diagnosis of von Willebrand's disease and for the detection of carriers of hemophilia; widespread application of home care programs in the management of hemophilia.

- Improved understanding of the mechanisms of action, indications for therapeutic use, and schedules of dosage of heparin and the oral anticoagulants.

- Evaluation of aspirin, dipyridamole, sulfinpyrazone, and combinations of these agents in clinical trials in arterial thrombotic diseases; identification of the limits of usefulness of antiplatelet therapy in advanced arterial thrombotic diseases.

In addition, several major technical advances of the past 10 years have impinged upon hemostasis and thrombosis research: recombinant DNA technology; monoclonal antibodies; culture methods for endothelial cells, megakaryocytes, and liver cells; techniques

for separating platelets in their native, unstimulated state; techniques for the purification of clotting factors that made kinetic studies of the blood coagulation reactions possible.

Needs and Opportunities

The following needs and opportunities can be identified for the next 5-year period:

Basic Research

- Apply recombinant DNA techniques to studies of the blood coagulation and fibrinolytic proteins in order, ultimately, to characterize the genomic DNA for each of the coagulation and fibrinolytic proteins.

- Stimulate studies, utilizing animal models and tissue culture systems, of the control of production and turnover of platelets and of the plasma proteins involved in blood coagulation and fibrinolysis.

- Increase understanding of blood vessel wall biology as it relates to normal hemostasis and to the pathogenesis of thrombosis and atherosclerosis, through:

  - Studies of the mechanisms by which endothelial cells maintain their nonthrombogenic properties

  - Investigations of the interaction of the subendothelium and its components with platelets and their constituents and with the blood coagulation and fibrinolytic factors.

- Encourage rheologic studies of hemostasis, including development of models to study platelet-vessel wall interactions and the effect of alterations of endothelial cell surface proteins upon rheologic parameters.

- Investigate, at the molecular level, the structural properties and biochemical reactions of platelets important for platelet adhesion and aggregation, including: interactions of von Willebrand protein with the platelets and vessel wall components; the structure and function of the platelet surface membrane glycoproteins; the platelet contractile mechanism; functions of cyclic nucleotides, prostaglandins, and other lipid-derived materials; functions of materials secreted from platelets.

- Continue structural analysis of the plasma coagulation proteins with the ultimate goal of determining for each protein: primary structure, three-dimensional structure, structural properties that determine its specific biochemical function(s) in blood coagulation, and the alterations of tertiary structure associated with its activation during blood coagulation.

- Expand studies of the reactions initiating, amplifying, and regulating blood coagulation from those limited to soluble system to studies involving reactions at cell surfaces and with cell surface components. Of particular importance are studies of:

  - Blood coagulation reactions at the platelet surface

  - Tissue factor: purification and structural analysis, location and organization within cell membranes, mechanisms determining its availability on cell surfaces, and kinetics of the tissue factor-factor VII initiation of coagulation on cell surfaces.

- Delineate further the function of protein C in the regulation of blood coagulation and fibrinolysis, and determine the hemostatic function, if any, of protein S.

- Define, in molecular detail, the biochemical reactions whereby each of the plasma protease inhibitors important for hemostasis inactivate the plasma proteases with which they react, including the manner in which specific ligands such as heparin and amines modulate reactivity.

- Broaden understanding of the mechanisms initiating and regulating fibrinolysis, including: factors controlling synthesis, release, and hepatic clearance of vascular plasminogen activator; the structural basis for the activity of vascular plasminogen activator and its increased affinity for plasminogen induced by denatured protein; further delineation of the structure-function relationships of plasminogen and plasmin; and further delineation of the molecular mechanisms by which alpha-2-antiplasmin and histidine-rich-glycoprotein modulate fibrinolysis.

- Broaden understanding of the links between hemostasis and other key biologic processes: inflammation, reactions of the complement system, cellular immune responses, and wound healing.

Clinical and Applied Research

Specific goals for research in hemophilia and acquired bleeding disorders that deserve particular emphasis in the next 5 years include:

- Elucidate why patients with hemophilia A develop factor VIII antibodies, develop methods preventing formation of such antibodies, and devise therapy to reestablish tolerance to factor VIII in patients now possessing such antibodies.

- Devise methods to improve yield in the preparation of factor VIII concentrates and to eliminate the thrombogenic materials from prothrombin complex concentrates.

- Apply the techniques of genome analysis of cultured amniotic tissue fluid cells to the problem of in utero identification of hemophilia and the carrier state.

- Continue evaluation of the techniques and results of modern management of hemophilia, including standardization of optimal schedules of factor VIII dosage, and further delineation of the clinical consequences of chronic liver disease and circulating immune complexes.

- Produce plasma concentrates for use in the less common hemorrhagic disorders, such as factor XI deficiency and factor V deficiency, and develop a means for making the concentrates available nationally.

Specific goals for research in the platelet disorders that deserve particular emphasis during the next 5 years include:

- Characterize the platelet antigens and the antigenic specificity of the antibodies arising in patients with idiopathic thrombocytopenic purpura.

- Elucidate the pathogenetic mechanism for thrombotic thrombocytopenic purpura and related syndromes, and standardize therapy for these disorders.

- Delineate the pathogenesis of bleeding in uremia, including identification of the plasma materials that impair platelet function, and standardize the management of uremic bleeding, including further evaluation of the therapeutic effectiveness.

- Improve platelet transfusion therapy through clarification of indications; improve methods of preparation and storage

of platelet concentrates; develop methods for pretransfusion cross-matching; and develop methods to prevent and detect immunization to platelet antigens and contaminating lymphocytes.

Specific goals for research in the thromboembolic disorders that deserve particular emphasis in the next 5 years include:

- Establish the importance and limitations of tests that reflect activation of platelets, such as PF4 and beta-thromboglobulin; activation of blood coagulation, such as fibrinopeptide A, and activation of fibrinolysis, such as fragment E, in the prevention, diagnosis, and treatment of thrombotic disorders.

- Continue studies of the properties and actions of heparin, and utilize that knowledge to prepare better heparin for clinical use.

- Develop and evaluate new drugs or plasma proteins for possible therapeutic use in the thrombotic disorders, including stable analogs of prostacyclin and inhibitors with specificity for one or more of the clotting or lytic proteases.

- Conduct a cooperative, prospective clinical study of the management of venous thrombotic disease with the goal of developing specific recommendations for:

  - The prophylactic use of low-dosage heparin

  - The time, after beginning heparin, that warfarin should be started

  - The indications for fibrinolytic therapy and the evaluation of its attendant risks

  - The period that anticoagulant therapy should be continued after an acute episode has subsided and the level of anticoagulation that is needed in long-term therapy.

General Recommendations

Continued scientific productivity of the order achieved during the past 10 years requires a steadfast determination to

maintain the far-sighted research policies and research management practices of the NIH that have resulted in:

- Strong programs at several levels for the development and support of biomedical investigators.
- Adequate levels of funding for investigator-initiated research, including funding sufficient to accommodate the beginning efforts of new investigators and sufficient to support unorthodox approaches to problems.
- Protection of the flexibility of research activity that allows optimal exploitation of new knowledge or chance observations.

Specific Recommendations

Targeted funds, for use either to stimulate investigator-initiated research or to support contracts, should be considered for the following purposes:

Basic Research

- Studies of tissue factor: structure, function, synthesis, and location in cell membranes.
- Studies of the interaction of blood vessel wall components and hemostatic factors.
- Studies of platelet and clotting factor production, utilizing megakaryocyte and liver cell culture methodology.

Clinical and Applied Research

- Studies of mechanism of development, prevention, and management of factor VIII antibodies in hemophilia.
- Production and distribution of plasma concentrates for use in the less common hemorrhagic disorders.
- Investigations of the pathogenetic mechanism and treatment of thrombotic thrombocytopenic purpura.
- Studies of the pathogenesis and management of bleeding in uremia.

- Improvement of platelet transfusion therapy.
- Development of better preparations of heparin for clinical use.
- A cooperative, prospective clinical study of the management of venous thrombotic disease.

Needs Unrelated to Specific Single Research Goals

- Maintenance of animal colonies (primates, hemophilic dogs, von Willebrand pigs) and the search for additional animal models needed for research on thrombosis and hemostasis.
- Replacement of equipment in established laboratories. Equipment is wearing out in many laboratories. Mechanisms should therefore be found for the retooling of laboratories.
- Purchase of very costly items of equipment necessary for particular areas of research, such as for prostaglandin research.

Red Blood Cells and Their Disorders

The erythrocyte, with its ready availability, simple structure, limited metabolic needs, and primary function of transporting oxygen to the tissues, has served as a premium model for basic, applied, and clinical research. During the last 10 years, studies of this anucleate cell have contributed most significantly to knowledge about human disease processes, how they are caused, and to some extent, how they may be modified. These investigations have enhanced the understanding of fundamental cellular and molecular biology, normal and abnormal cellular proliferation and its hormonal control, human genetics, the pathogenesis of inherited and acquired diseases (especially anemia), and the treatment of disorders of decreased erythrocyte production (aplastic anemia, vitamin B_{12}, folic acid, and iron deficiency) and increased destruction (acquired and inherited hemolytic anemias).

The frequency of some of the erythrocyte disorders is high and worldwide (iron deficiency), and the frequency of others is fortunately rare and often restricted to certain populations. Some of the disorders, such as sickle cell anemia, thalassemia major, and aplastic anemia, are debilitating and ultimately fatal. Because of their chronicity, they exert a disproportionate burden on patients, their families, and society. Appreciation of the

anemias that result from vitamin B_{12} and folic acid deficiencies has enhanced the diagnosis and simplified the treatment of these diseases, but such nutritional deficiencies often accompany other illnesses and are overlooked or ignored. The acquired hemolytic disorders that can accompany other diseases, such as malignancies or collagen vascular disorders, compound the care of afflicted patients. Thus, while primary disorders of the erythrocyte may not lead to statistically dramatic death rates, they contribute very significantly to morbidity, with resultant losses in the quality of life, reductions in life span, and increases in costs of necessary and appropriate medical care.

Major Advances of the Past 10 Years

During the last 10 years, dramatic contributions to knowledge about the erythrocyte and its primary component, hemoglobin, have led the way to the initial application of the technologies of molecular and cell biology to the understanding of human disease. These accomplishments are summarized below under five major headings: hemoglobin, sickle cell anemia, the thalassemias, erythrocyte membrane and metabolism, and erythropoiesis.

Hemoglobin

- Elucidation of the three-dimensional structure of human hemoglobin and of the mechanisms underlying its physiologic behavior. Delineation, at the level of structure and physiology, of the interactions between hemoglobin and the two most important physiologic modifiers, hydrogen ions and organic phosphates (2,3 DPG). The formation of allosteric models that explain functional properties of hemoglobin.

- Discovery that hemoglobin is useful as a reporter molecule that reflects environmental fluctuations in health and disease and specifically the recognition of usefulness of measurements of glycosylation of hemoglobin in monitoring patients with diabetes.

- Elucidation, through studies of hemoglobin, of the mechanisms of protein synthesis. Delineation of various factors that are necessary for protein synthesis as well as of functions of modifications of these factors in the control of protein synthesis.

- Delineation of the organization of globin genes in man and in several animals. Discovery of globin pseudogenes that

are homologous to functional genes but that are inactivated by mutation. Discovery within the globin gene clusters of several families of repetitive DNA sequences and their structural characterization.

- Elucidation of the exact nucleotide sequences of the globin genes. The discovery that each globin gene is composed of exons (that is, sequences that specify the structure of a globin protein) separated by two intervening sequences (introns). Discovery that the entire gene, including the introns, is copied in mRNA but that the intervening sequences must be removed to generate a functional globin mRNA.

- Definition of the structure of the globin gene promoter (the site at which DNA polymerase binds and globin gene transcription begins). Identification of DNA sequences important for efficient processing of globin mRNA.

- Development of systems designed to study globin gene transcription in cell extracts and within living cells.

- Discovery that the structure of chromatin at the globin genomic regions has a critical importance in globin gene expression. The discovery that modification of DNA at the globin genomic region is related to the expression of globin genes.

- Development of techniques for transferring globin genes into living cells. The use of these techniques for identification of DNA elements essential for globin gene function.

- Development of culture techniques and animal models that allow investigation of the regulation of the synthesis of fetal hemoglobin. The discovery that the production of fetal hemoglobin is controlled in stem cells and erythroid progenitors. The development of human established cell lines that are useful to the study of the cell biology of human hemoglobin switching.

The spectacular advances made in learning about the primary, secondary, tertiary, and quarternary structure of normal and abnormal human hemoglobin and about the location and function of the genes involved in the manufacture of the different protein polypeptide chains have clearly enhanced the understanding of the pathophysiology of various disease states and permitted initial efforts in modifying the deleterious effects of some of these mutations.

Sickle Cell Anemia

- Advancement in the understanding of the structure of the sickle cell polymer. Definition of specific areas of contact between deoxy Hb S molecules. Appreciation that stereospecific reagents binding to these contacts can be designed to serve as antisickling agents of potential clinical use.

- Development of techniques that allow investigation of the kinetics of polymerization of Hb S molecules. Advancement in understanding the mechanism of polymerization, and the development, through studies of polymerization, of new concepts regarding therapeutic approaches to sickle cell disease.

- Discovery of red cell membrane abnormalities in sickle cell disease and of the apparent abnormal adherence of SS red cells to vascular endothelium. Recognition that drugs that inhibit the appearance of such membrane abnormalities may carry therapeutic implications in sickle cell anemia.

- Development of molecular techniques, based on restriction endonuclease mapping, that allow the recognition of the abnormal codon in the Hb S gene. Application of these techniques in prenatal diagnosis of sickle cell anemia. Development of nearly 100 percent accurate tests that allow prenatal diagnosis using cells obtained through amniocentesis.

- Recognition of the importance of infections in sickle cell anemia. Recognition that mortality and morbidity can be reduced through early diagnosis of sickle cell anemia in newborns and through the control of infection in infancy and childhood.

- Systematic effort to define the natural history of sickle cell disease and derive quantitative parameters for use in the management of patients and in the evaluation of the clinical effects of antisickling agents or other therapeutic approaches.

- Development of programs of comprehensive care of sickle cell patients and the delivery of this care through sickle cell centers.

The insights that have been gained into the natural history of sickle cell anemia and the comprehensive programs to foster education, genetic counseling, and improved patient care have been successfully combined with basic research to advance knowledge

about this hemoglobinopathy. Modern technology has made increasingly accurate prenatal diagnosis possible where desired or required. Although not of themselves successful, efforts to modify the clinical course of sickle cell anemia by the introduction of antisickling agents have led to an improved understanding and appreciation of this disease and to improved care for those who are afflicted.

The Thalassemias

- Delineation of the molecular pathology of the alpha-thalassemia syndromes. Discovery that alpha-thalassemia in Orientals and blacks is due to deletions of one or more alpha-globin genes. The discovery that 30 percent of the American black population has a deletion of one alpha-globin gene.

- Delineation of the molecular pathology of the beta-thalassemia and delta, beta-thalassemia syndromes. Discovery that delta, beta-thalassemias and certain beta-thalassemias are due to globin gene deletions. Recognition that certain beta-thalassemias are due to nonsense mutations that result from single nucleotide base substitutions in the beta globin genes. Discovery that mutations that affect the processing of messenger RNA also underlie several forms of beta-thalassemia.

- Application of molecular technology in prenatal diagnosis of homozygous beta-thalassemia (Cooley's anemia), and development of accurate restriction endonuclease mapping procedures that allow prenatal diagnosis of the majority of Cooley's anemia homozygotes.

- Development of hypertransfusion regimens for treatment of patients with Cooley's anemia. Development of a treatment of the iron overload in this condition through semicontinuous subcutaneous infusion of deferoxamine. Application of this therapeutic regimen in medical practice.

Elucidation of the genetics of the thalassemic disorders, although still incomplete, has significantly improved the ability to diagnose the heterozygous carrier state and to initiate accurate prenatal diagnosis. Therapeutic interventions, including the administration of transfusions especially of erythrocytes of younger average age, and the use of iron chelators to remove potentially harmful iron, have prolonged the life of patients affected with beta-thalassemia (Cooley's anemia) and improved its quality.

Erythrocyte Membrane and Metabolism

- Advancement in understanding the structure and function of red blood cell membranes. Development of the concept of the skeletal proteins as the structural framework of the membrane. Advancement in understanding the regulation of ion movements by membrane components. Development of sensitive methods that allow investigation of membrane structure and function.

- Discovery of membrane protein abnormalities in disorders of cell shape and deformability associated with certain hereditary hemolytic anemias.

- Delineation of the regulation of glycolysis in the red cell. Detection of new enzymopathies associated with hereditary hemolytic anemias. Discovery that certain red cell enzyme deficiencies influence the oxygen-carrying capacity of the red cell by affecting the concentration of glycolytic intermediates.

- Discovery, through studies of red cells, that severe deficiencies of enzymes of purine and pyrimidine metabolism such as adenosine deaminase, nucleotide phosphorylase, or pyrimidine-5'-nucleotidase are the underlying abnormalities in certain congenital disorders of the immune system and in certain hereditary hemolytic disorders.

- Delineation, through biochemical studies of enzymes or of metabolic intermediates in the normal red cells and in red cells of patients with enzymopathies, of metabolic parameters of the red cells that are crucially important for the survival of these cells. Utilization of this knowledge for deriving conditions that allow prolongation of red cell preservation in vitro. Practical application of this knowledge for preservation of blood used for transfusions.

- Discovery that several inborn errors of metabolism that produce abnormalities in various tissues of the body are also reflected in deficiencies of the affected enzymes in the red cells. Utilization of this information for the diagnosis of diseases, detection of heterozygous carriers, and genetic counseling.

- Advancement in understanding the biochemical characteristics of red cell antigens. Advancement in understanding the biochemistry of the reactions of complement, and

appreciation of the importance of these reactions in the immune destruction of the red cells.

- Near eradication of hemolytic disease of the newborn by prophylactic administration of hyperimmune anti-D.

- Development of improved techniques for the qualitative and quantitative detection of antibodies and complement on the red cells, and the use of these techniques in the diagnosis of specific causes of immune hemolysis.

The partial elucidation of the complex protein structure of the erythrocyte membrane has permitted better insight into the pathogenesis of certain hereditary hemolytic disorders, the transport of water and iron into and out of the erythrocyte, and the maintenance of the structural integrity of the cell. Initial efforts to correlate alterations in the erythrocyte cytoskeleton with changes in the rheological properties of the affected cell have suggested the mechanisms involved in the premature destruction of erythrocytes in hereditary elliptocytosis and pyropoikilocytosis, and perhaps in hereditary spherocytosis.

Erythropoiesis

- Development of methods that allow culture of erythroid progenitors and of pluripotential stem cells. Detection of several classes of erythroid-committed progenitors, and the investigation of their characteristics in health and disease.

- Advances in the purification and biochemical characterization of the hormone erythropoietin and in understanding its importance in the regulation of erythropoiesis.

- Discovery of new regulatory molecules that influence the proliferation of stem cells and erythroid progenitors. Discovery that cellular components (such as T cells or monocytes or bone marrow stromal cells) are involved in the processes that regulate hematopoiesis.

- Advancement in understanding the pathogenesis of aplastic anemia. Definition of environmental factors that can cause this disorder. Appreciation that various abnormalities in the cells, in their environment, and in the immune response may cause aplastic anemia.

- Successful application of bone marrow transplantation in the treatment of aplastic anemia. Emergence of objective

criteria for selecting patients for bone marrow transplantation in this disorder.

- Advancements in understanding the pathogenesis of polycythemia vera and the establishment, through cooperative studies, of uniform criteria for diagnosis. Demonstration that polycythemia as well as other myeloproliferative disorders are clonal stem-cell defects.

The improved understanding of the function of the pluripotential stem cell and the erythroid progenitor cell, as well as of the microenvironment of the bone marrow, in the pathogenesis of aplastic anemia has led to the successful transplantation of bone marrow in certain selected cases.

Needs and Opportunities

During the next 5 years, opportunities are legion for advances in the understanding of erythrocyte pathophysiology that will lead to improved diagnosis and treatment. Research in the following areas shows promise of enhancing human well-being, preventing preventable illnesses, and furthering fundamental knowledge of basic biological processes. Because the areas are so intimately interrelated, basic, clinical, and applied research are not subdivided.

Hemoglobin

- Continue the delineation of the organization and expression of the globin genomic regions with emphasis on the discovery of regulatory elements. Develop efficient in vitro assays to allow testing of the function of molecular structures.

- Delineate the relationship between chromatin organization and globin gene expression. Identify the chromosomal proteins that are involved in the regulation of globin genes. Identify and characterize the DNA sequences in which these chromosomal proteins bind. Develop systems which allow investigation of globin gene transcription at the level of chromatin.

- Develop physiological and practical means for the introduction of globin genes into stem cells. Develop assays to allow testing of the function of the transferred genes. Develop methods allowing selection of the stem cells in which the globin genes have been introduced. Develop in vivo experimental systems in which the efficacy of the

globin gene transfer technology can be tested in preparation for its use for gene therapy of patients with thalassemia and sickle cell disease.

- Simplify the technique of globin gene analysis that uses restriction endonuclease mapping in medical diagnosis.

- Elucidate the molecular regulation of fetal hemoglobin synthesis with DNA sequencing and chromatin studies of those genetic defects that are associated with continuation of fetal hemoglobin production in the adult.

- Delineate the cellular mechanisms for the regulation of fetal hemoglobin synthesis in man with studies of fetal hemoglobin synthesis in cultures of hematopoietic cells; with observations in humans who seek medical aid for elevated concentrations of fetal hemoglobin; and with investigation of animal models in which production of fetal hemoglobin can be induced.

- Search for harmless and practical means for the induction of fetal hemoglobin synthesis in human adult individuals, and develop approaches for treatment of sickle cell anemia and Cooley's anemia through induction of fetal hemoglobin production.

- Complete the delineation of the molecular relationship between structure and function of the hemoglobin molecule. Delineate the function as well as the interactions of hemoglobin with other molecules under the conditions of high hemoglobin concentration prevailing inside the red cell. Delineate the mechanisms of hemoglobin assembly and degradation within the red cell and the mechanisms of hemoglobin modification in various hematopoietic and nonhematopoietic disorders.

Sickle Cell Anemia

- Complete the elucidation of the structure of the hemoglobin S (Hb S) polymer. Establish with certainty the number and arrangement of the Hb S strands in this polymer. Determine the amino acid residues involved in the intermolecular contacts between strands. Design stereospecific inhibitors of sickling based on the information obtained from the structural studies of intermolecular contacts.

- Elucidate the in vitro and in vivo mechanism of polymerization of Hb S through high resolution kinetic

approaches. Use the results of these kinetic studies in order to devise therapeutically useful approaches for inhibition of Hb S polymerization.

- Expand the study of the sickle cell membrane to focus on determining the contribution of the sickle cell membrane to the in vivo sickling process, on delineating the mechanisms responsible for the interaction of sickle cell membranes with the endothelial surface of capillary vessels, and on developing membrane-active drugs that interfere with in vivo sickling.

- Advance the understanding of the behavior of sickle cell erythrocytes in the circulation of patients through research on the rheology of sickle cells. Devise noninvasive ways of studying blood flow in sickle cell patients. Use these methods for studying the cellular pathophysiology of sickle cell anemia and for evaluating potential therapeutic agents.

- Develop antisickling agents without adverse side effects. In addition to stereospecific inhibitors of sickling and membrane-active compounds, explore approaches to diminish the intracellular concentration of sickle hemoglobin and to allow simple and safe extracorporeal delivery of antisickling agents.

- Devise harmless methods allowing induction of fetal hemoglobin synthesis in sickle erythrocytes in vitro. Test the efficacy of these methods in experimental animals. Attempt induction of fetal hemoglobin synthesis in the sickle cell patient.

- Advance the understanding of pathophysiology of sickle cell disorders. Resolve the physiological consequences of Hb S to the red cell, to the microvasculature, and to organ function. Identify the genetic or environmental parameters that modify the course of sickle cell anemia, and define the importance of these parameters in modifying the behavior of sickle cells.

- Delineate the mechanism of the sickle cell crisis. Understand the factors that precipitate a crisis, and develop means allowing objective diagnosis and quantification of the severity of a crisis.

- Delineate the natural history of sickle cell disease, and devise objective criteria for the evaluation of the severity of this disorder. Accumulate the basic clinical

data required for the evaluation of the results of clinical trials of antisickling agents.

- Test the efficacy of therapeutic modalities including the effect of systematic long-term transfusion treatments, the possible beneficial effects of anticoagulants, and alternative forms of management of patients with special problems or complications such as pregnancy.

- Develop a mechanism through which the advances in prenatal diagnosis of sickle cell disease through the application of molecular techniques become available to the population at risk.

The Thalassemias

- Delineate the molecular pathology of the thalassemia syndromes with studies of the fine structure of thalassemia genes, studies of mRNA synthesis and metabolism, investigations of the molecular lesions leading to mRNA processing defects, and investigations of the expression of thalassemia genes in cellular or cell-free transcription systems in vitro.

- Develop an iron chelating agent with either an oral route of administration or a long in vivo half-life, so that treatment of patients can become less cumbersome and less costly.

- Improve the currently available modalities of transfusion treatment of the thalassemia syndromes.

- Explore the therapeutic usefulness of bone marrow transplantation in patients with severe homozygous beta-thalassemia (Cooley's anemia).

- Develop a mechanism through which prenatal diagnosis of thalassemia syndromes with the use of molecular techniques becomes available to the population at risk.

- Experiment on gene therapy of the beta-thalassemias with studies of the transfer of normal globin genes into hematopoietic cells in vitro and in suitable animal models.

- Understand the cellular and molecular mechanisms of regulation of fetal hemoglobin synthesis in man. Develop in vitro methods allowing stimulation of fetal hemoglobin production in thalassemic cells. Devise approaches that

permit induction of fetal hemoglobin production in animals and eventually in patients with Cooley's anemia without causing harmful effects elsewhere in the body.

Erythrocyte Membrane and Metabolism

- Delineate the structure and function of the red cell membrane cytoskeleton and the importance of the interactions between skeletal proteins. Elucidate the contribution of the red cell membrane to normal and abnormal red cell survival. Delineate the mechanism of the coordinated assembly of membrane proteins of the red cell. Elucidate the importance of red cell membrane modifications such as glycosylation and phosphorylation and their alterations by somatic disorders.

- Explore the use of the red cell membrane as a diagnostic tool in diseases such as hypertension, obesity, and other potential membrane-mediated disorders. Explore the utility of red cell enzyme studies in the diagnosis of acquired hereditary metabolic disorders.

- Identify and isolate the genes responsible for the synthesis of membrane proteins.

- Support research on the pathogenesis of hereditary and acquired hemolytic anemias. Only a few of the congenital hemolytic anemias have been biochemically elucidated.

- Elucidate red cell metabolism and the process of production, maintenance, and decay of red cell enzymes and of those critical metabolic intermediates that assure the normal survival of red cells in vivo. Use the understanding of red cell metabolism in studies focused on improving blood preservation.

- Elucidate the processes that initiate autoimmune hemolytic syndromes, and develop improved techniques for the detection of immunochemical reactions of the red cell.

Erythropoiesis

- Use monoclonal antibodies to isolate and characterize pluripotential stem cells and erythroid progenitors. Elucidate the kinetics of stem cell and progenitor cell proliferation and differentiation.

- Elucidate the characteristics of the hematopoietic factors. Purify factors such as erythropoietin and burst-promoting activity, and delineate their biological function. Search for factors unrecognized but suggested by studies of hematopoietic cell interactions. Delineate the physiological relevance of hematopoietic factors. Examine the therapeutic potential of these factors in disorders of erythropoiesis.

- Develop culture systems allowing efficient proliferation of pluripotential stem cells and of erythropoietic progenitors. Use these systems for elucidating the regulation of normal erythropoiesis as well as the pathogenesis of disorders of erythropoiesis. Use pluripotential stem cell cultures for in vitro studies of globin gene transfer. Evaluate the possibility of using improved cultures of stem cells for obtaining the number of cells required for bone marrow transplantation and for bypassing the problem of graft versus host disease.

- Support clinical and biological studies of the pathogenesis of disorders of erythropoiesis such as congenital hypoplastic anemias or aplastic anemias. Search for therapeutic modalities of these disorders in the light of knowledge gathered through studies of their pathogenesis.

- Support research to simplify in vitro hematopoietic cell assays for their eventual routine application in diagnosis as well as for the treatment of disorders affecting hematopoietic stem cells and erythroid progenitors.

Recommendations

Studies of the erythrocyte during the past decade have amply demonstrated the intimate relationship between molecular biology, cell biology, biophysics, classic biochemistry, modern genetics, and clinical medicine. The erythrocyte readily lends itself to studies designed to answer some of the vexing problems of human disease. Although diagnostic acumen has been heightened dramatically, the process of moving to the bedside the answers found in the laboratory has only begun. A prime recommendation, therefore, must be to continue to enhance these relationships to permit full exploitation of the very real possibilities of preventing and modifying the debilitating and costly illnesses that arise as the result of inherited or acquired abnormalities of the human erythrocyte.

These objectives can best be achieved through the support of investigator-initiated research, supplemented by well-organized

and focused program projects. Helpful guidance may be provided through the identification of areas of neglect or fields ready for development. Essential to the foregoing is the maintenance of adequate research training programs for the next generation of biomedical scientists with either MD or PhD degree, as well as of carefully defined and well-funded research grant opportunities for new investigators in transition from postdoctoral research training to established investigative careers.

Areas for research emphasis in the next 5 years are:

- Acquisition of additional detailed information about the genes involved in the regulation of the synthesis of normal and abnormal human hemoglobins, the mechanisms by which fetal hemoglobin synthesis is controlled, stopped, and started; and the techniques essential for the eventual manipulation of genetic coding, gene activation, and gene regulation.

- Specific information about the primary, secondary, and tertiary structure of the erythrocyte skeletal membrane proteins and about the genes responsible for their synthesis.

- Additional information about the metabolic processes of mature human erythrocytes and their regulation that would permit enhancement of survival of abnormal erythrocytes in vivo and the preservation of normal erythrocytes in vitro.

- The development of a ready source of pure erythropoietin, and purification and characterization of other hematopoietic cellular growth factors, such as burst-promoting factor and colony-stimulating factor.

- Clinical research on sickle cell disease and the thalassemias to explore the feasibility of inducing fetal hemoglobin synthesis; on chemical interventions to modify the adverse properties of sickle hemoglobin; on bone marrow transplantation, if the safety and efficacy of the procedure can be clearly established so that the treatment will not be worse than the disease; and on genetic engineering of hemoglobin genes, but only after thorough and completely convincing demonstrations in experimental animals and in in vitro systems.

Blood Resources

The Division's involvement in the blood resources area has undergone marked and important changes during the period encompassed by this review. In 1972, a major emphasis was directed toward establishing a national blood policy that would assure the citizens of this country a ready supply of safe, high-quality blood. General objectives, such as voluntarism, regionalization, commonality, and data collection were discussed at great length. The private sector was encouraged to pursue these objectives with the help of financial and programmatic support from the government.

A second facet of the 1972 National Program relating to blood resources consisted of a number of research oriented, basic science objectives. Goals included developing better techniques for preserving, for prolonged periods, cellular components of the blood by learning more about the factors responsible for viability and function. New methods for protein fractionation were projected so that blood derivatives of better quality would be available for clinical use. New protein fractions, some with poorly understood physiologic functions, were also to be sought and studied. Finally, a concern for safety, in terms of the transmission of disease as well as in terms of safety for donors and recipients, was strongly expressed.

These objectives have been generally met. In addition, new research directions have emerged in the blood resources area as a result of the development of new techniques and the maturation of thinking concerning the nature of the discipline itself. New techniques have had a significant impact on a field that is concerned primarily with product development and technological advances. The result has been the introduction of new agents and reagents and the application of new, sophisticated instrumentation to problems of blood collection and treatment of disease. Within the discipline, clearer definitions of the scope of the blood resources area have emerged. Not only are investigators in transfusion medicine concerned with cellular preservation and component preparation; new responsibilities involve such widely divergent subjects as oxygen-carrying substitutes, therapeutic apheresis, and the optimal management of the national blood resource.

Also emerging in the course of the decade has been an appreciation of the magnitude of the field covered by the term "blood resources." Involving populations of 5 to 6 million individual voluntary donors and of about 3 million patients who are recipients of blood and major blood products, the field of transfusion medicine directly affects 4 to 5 percent of the U.S. population. Added to these statistics is the major impact that

newly emerging techniques, such as therapeutic apheresis, are likely to have. Therapeutic apheresis may be applicable to as many as an additional one-half million people suffering from a range of rheumatoid, immune, and neurological disorders. Application of these new techniques could have a major economic impact.

Major Advances of the Past 10 Years

Major advances have taken place during the past 10 years in most of the areas of concern to those interested in transfusion medicine. A number of these advances could not have been predicted in 1972 because the techniques for pursuing them were not then available. Listed below are key developments:

- Improvements in the quality and use of blood transfusions have been achieved by
  - Conversion to an almost 100 percent voluntary blood donor system
  - More effective inventory management, aided by the development of new preservatives extending the shelf-life of blood and helped by the refinement of the concept of resource sharing
  - Gradual acceptance of the concept of component therapy for specific medical indications spurred on by the availability of a range of effective blood products
  - Reduction in the number of adverse consequences of blood transfusions as a result of improved laboratory methods and a better understanding of the pathophysiology involved.
- Understanding factors affecting red cell viability and function, such as pH, ATP levels, and 2,3-DPG concentrations, has permitted striking improvements in the ability of blood banks to preserve functioning erythrocytes for prolonged periods of time in both liquid and frozen states. The development of blood-compatible plastic materials, preservatives such as CPDA, and new methods of rejuvenating stored red cells have contributed to these improvements.
- Knowledge about the structure and function of the red cell membrane has led to the development of better immunologic methods for assuring blood compatibility; to an appreciation of the importance of complex antigenic determinants

in the erythrocyte membrane in maintaining normal physiology of the red cell and to the development of pathology when altered; and to the discovery of methods for manipulating antigenic sites on the membrane that make it possible to prepare red cells with specific immunologic characteristics.

- The availability of functional platelet preparations and the development of techniques to preserve them for 3 to 5 days has had a major impact on clinical conditions characterized by low platelet counts, particularly those situations associated with modern aggressive cancer chemotherapy. Important factors in this progress were the introduction of automated cell separators, which can be used to obtain large quantities of single-donor platelet preparations, basic research studies leading to an understanding of the metabolic machinery of the platelet and the means for preventing rapid deterioration in vitro of this capability, and the development of an appreciation of platelet immunology in relation to compatibility and immunization.

- While methods for preserving granulocytes have not kept pace with techniques available for maintaining other blood cellular elements, important progress has been made in harvesting large quantities of leukocytes. These developments involve the use of automated instrumentation and the stimulation of granulocyte yields through the use of appropriate premedication. The clinical value of granulocyte transfusions has been demonstrated in specific instances of low white counts and accompanying gram-negative sepsis.

- Specific white cell components have been investigated as sources of important compounds, such as interferon and transfer factor. In addition, new culture techniques, which allow in vitro growth of subclasses of white cells, including stem cells, provide material for research, for use in developing monoclonal reagents, and for analyzing the immunologic complexity and physiologic function of the many lymphocyte populations.

- Purified plasma proteins are readily available today as a result of improved technology developed during the last decade. Included are not only albumin and the immunoglobulins, but also well-characterized clotting factors, such as VIII and IX, and a large number of recently described agents that contribute to health and disease, such as the plasma proteinase inhibitors, enzymes, and binding proteins. The availability of these substances in

quantity has allowed investigators to purify some to homogeneity, determine important structural characteristics, and understand the functional importance of these substances.

- The availability of antihemophilic factor concentrates and other products containing purified or activated clotting proteins has revolutionized the treatment of hemophilia and made it possible to help patients with von Willebrand's disease, factor VIII inhibitors, factor IX deficiency, and hereditary and acquired deficiencies of the vitamin K-dependent clotting factors.

- With improved techniques, it became possible to isolate stable preparations of many plasma proteins from source plasma, including the immune globulins, free of trace amounts of substances that cause adverse reactions. One result of this advance is the recent introduction of an intravenous immune globulin preparation that is likely to be of great clinical value.

- Several oxygen-carrying blood substitutes were investigated during the decade. Preliminary clinical trials are underway evaluating the efficacy of stroma-free hemoglobin solutions and perfluorocarbon suspensions in the acute treatment of blood loss. Basic laboratory research suggests that both may make important contributions to medical care, particularly in medical emergencies.

- The control of posttransfusion hepatitis (PTH) has been one of the remarkable outcomes of basic research on the epidemiology and serology of the agents responsible for the disease. In addition, technological advances, as represented by sensitive specific tests for some of the causative agents of PTH, particularly hepatitis B virus (HBV), have resulted in a major reduction of the disease in this country. Newer achievements include the development of an effective vaccine against the hepatitis B virus that promises to have a major impact on the epidemiology of this disease throughout the world. Each of these advances represents an important improvement in cost-benefit ratio in terms of the research dollar invested and the reduction in health care and allied costs.

- In spite of major advances in the control of hepatitis A and B viruses, most PTH is now caused by an unidentified agent(s), called non-A,non-B. These agents, as is true with HBV, can be propagated in the chimpanzee. An important step in the eventual control of this virus will be the availability of a large bank of sera, collected

prospectively, from donors and patients receiving blood transfusions, some of whom later developed PTH.

- Major technical advances in plasmapheresis have encouraged the applications of this technique to therapeutic problems. Preliminary results of therapeutic apheresis in a number of conditions associated with immune complexes are encouraging, but controlled clinical trials are required to evaluate the approach.

Technological developments that were critical to this progress supported many of these advances: biocompatible plastics, effective separation techniques using columns, immunoabsorbants and automated instrumentation, monoclonal antibodies, and recombinant DNA techniques.

Needs and Opportunities

For the coming 5-year period, the review panel projects a shift from the emphasis of 1972 because the national blood policy of a decade ago to a large extent has been fulfilled. While research into several aspects of blood bank management and data accumulation continues to be desirable, these objectives are largely the responsibility of the blood banking industry in general, of the various professional blood bank organizations in particular, and of the governmental regulatory agencies charged with such tasks.

The focus of the Division in this decade must be on efforts to investigate the many unsolved scientific problems that dominate the field. Support for specific efforts is needed, but as experiences of the last decade demonstrate, advances often come in entirely unexpected and novel areas of research. Support of basic investigation continues to yield significant results. Furthermore, the level of maturity and sophistication that the field of blood resources has achieved suggests that it may now be time to develop three or more specialized centers of research in blood resources where research, pilot production, and clinical investigation into these products can be effectively pursued.

The following needs and opportunities can be identified in the next 5 years:

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.

- 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.

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.

- 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.

- 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.

- A large number of substances are actively transported on, or secreted by, cellular elements. Some are recognized to be of significant physiologic 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 monospecific antibodies and other techniques

and the application of recombinant techniques to produce the substances in useable quantities should be undertaken.

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.

- Specifically needed in this area 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.

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, which is now the prime cause of this disease. Efforts to minimize the occurrence of cytomegalovirus and other less common agents as a cause of hepatitis, particularly in certain specialized patient populations, must be continued.

- 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.

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.

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 tissues with monoclonal antibodies.

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.

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.

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.

General Recommendations

Keeping in mind that major advances in the field of blood resources occurred in areas not mentioned at all in the National Program of 1972 or referred to only in passing, one should reemphasize the statement that the support of basic and applied research in the manner practiced by the NIH in the past decade is highly desirable. Furthermore, the carefully planned programs for training and developing research investigators must be maintained. Central to both these activities are continuity, commitment, and flexibility on the part of the granting agencies in order to assure continued progress by productive investigators.

Specific Recommendations

Investigator-initiated research, stimulated when necessary by Institute initiatives and set-aside funds, should be considered for the following purposes:

Basic Research

- Studies of the metabolism of the storage defect of erythrocytes, granulocytes, and platelets.
- Studies of the immunology of cellular membranes.
- Studies of the structure, function, synthesis, and location of substances carried or secreted by blood cells.
- Studies to develop more effective techniques for separating plasma factors involved in clotting, immune function, inflammation, and other body defense systems.
- Studies of the epidemiology and serology of viruses transmitted by blood and blood products.

Clinical and Applied Research

- The development of more effective preservatives and rejuvenation solutions for long-term liquid and frozen preservation of formed elements of blood.

- Studies of cell culture techniques that will permit "test tube" marrow to be developed.

- Studies to free blood components from contaminating viruses that cause hepatitis and other diseases.

- Development of vaccines to immunize the population against non-A,non-B hepatitis.

- Development of improved blood substitutes to carry oxygen and provide oncotic pressure in emergency situations.

- Controlled clinical trials to test the efficacy of therapeutic apheresis procedures.

- Development of improved instrumentation and equipment for more efficient blood collection, reduced opportunity for bacterial contamination, and increased safety of donors.

Other Needs

- The development of a program to advance the teaching and recognition of transfusion medicine as a legitimate discipline in medicine is desirable. Such a goal may be achieved by the initiation of an Academic Award in Transfusion Medicine under NHLBI auspices.

- As a result of the maturation of the discipline of transfusion medicine and of the increasing complexity of the field of blood resources, specialized centers for basic, clinical, and applied research in the development, testing, and use of techniques and products relevant to the discipline are needed. A special effort should be made to establish three or more such centers in the next 5 years.

- Continued support of special facilities and resources important to research in the blood resources area is mandatory. Included are animal facilities, specimen banks, and data collection facilities. Resource development is required in tissue culture work, in pilot-scale separation and production equipment, and in sophisticated instrumentation.

- Clinical trials based on statistically sound, controlled, well-conceived protocols are needed in several areas of transfusion medicine.

- Registries may be needed for HLA-typed donors of platelets, granulocytes, and bone marrow. Efforts should be made to initiate appropriate registries to facilitate the optimal use of these scarce resources.

Prevention

The term "prevention" refers to precautionary measures that block or hinder the occurrence or progress of something, and it is perhaps the single most comprehensive term for summarizing all the activities that comprise the mission of the National Institutes of Health: to promote health by preventing diseases or by curing diseases that cannot yet be prevented. The term has come to be used also to refer to a category of activities--particularly those related to education, intervention, and control--that imply a separation from basic and clinical research. It should be restated that success in preventing diseases comes from an understanding that comes only from fundamental research and its ultimate contribution to the rationale for clinical studies.

The initial direct involvement of the Federal Government in prevention activities was the first Federal Quarantine Act of 1878, through which the Congress appropriated funds "for investigating the origin and causes of epidemic diseases, especially yellow fever and cholera." Over a hundred years later, the primary health initiative of the Secretary of Health and Human Services is in prevention, with the theme of "health promotion." The activities that the Secretary lists* as supporting this theme include basic and applied research, education of the public and of health-care professionals, specific service programs that encourage preventive measures, dissemination of information, and technical assistance to state and local governments and to the private sector. Specific activity proposed by the Secretary for the NIH includes the identification of discrete research topics that hold promise for health promotion and the development of a proposal for improving the application of findings related to health promotion. In implementing the Secretary's initiative, the Acting Director of the NIH has stated that there is general agreement among the Directors of the NIH Institutes that the major contribution of

*Memorandum: "Health Promotion Executive Committee," from the Secretary of Health and Human Services, March 18, 1982.

this agency is to perform research in disease prevention and the physiologic basis of health, and that the NIH will continue efforts to provide practitioners with new scientific knowledge that can be effective in the prevention of disease and disability. This recognition of the prevention-oriented strengths of the NIH continues to be the guiding philosophy of the Division of Blood Diseases and Resources.

Research Training and Development

Since the creation of the Division in 1972, research training support has been a high priority. The DBDR is now the major national source of funding for training in hematology. Two of every three investigators projecting an involvement in the area of blood diseases have received some or all of their training under the auspices of the DBDR. In addition, almost 100 percent of trainees involved in the blood transfusion sciences have been supported by the Division.

A number of concerns that have surfaced during the past 10 years deserve comment. Although inadequate stipends and the "payback" provisions may have adversely influenced recruitment, the Division's experience with the payback provision does not appear to be wholly negative. The inability to recruit MD's into research, which is apparently an important issue in many medical disciplines, does not appear to have been a major concern in hematology. Retention of the physician as an active investigator, however, has emerged as a significant problem. This inability occurs for a number of reasons, many of which are outside the control of the NIH.

Estimates of numbers of investigators needed in the various special areas of hematology are difficult to develop, but prospective data can be collected on which to base such projections. Those who are actively engaged in academic hematology suggest that shortages of personnel exist in basic disciplines related to coagulation and in most subjects of interest to blood transfusion scientists. An active pool of 300 to 500 trainees, only one-half of whom will complete their training and remain in research, is needed to maintain the present research effort in hematology.

Specific recommendations to improve recruitment and retention of young scientists for careers in basic and clinical research include the following:

- Opportunities for training should be provided early in the career of young scientists so that the commitment to a life of investigative activity is made easier. This goal

is particularly important in the case of the medical student, for whom other career choices are readily available.

- These opportunities may require new NIH training programs and cooperation on the part of those responsible for medical school curricula so as to allow a "year-out" training experience combined with some assurance that both postdoctoral research training and modest initial research support will be available.

- Creative relationships between the NIH, health foundations, and the public sector to ensure stability of training opportunities and the most effective use of financial resources for this purpose should be explored.

- An ongoing working committee concerned with issues of training should monitor developments in this area and make recommendations for the future. Adequate representation from government, academia, and industry is needed.

Conclusions

The most important lesson to be learned from the massive effort resulting in this report is that "if the thing isn't broken, don't fix it." Traditional investigator-initiated research has proven to be the most effective means of developing new scientific information, the latter often emerging in completely unexpected ways. Esoteric basic research has a way of bringing new insight into problems of major clinical importance. Directed research has led to significant new understandings.

For investigators to pursue active careers in research, they must have an opportunity to compete for adequate financial support. Recognition of the increased cost of research, the genuineness of the cost of research to the sponsoring institution, and the increased sophistication of research today, which allows many more complex problems to be approached, should lead to more appropriate support for biomedical research. Stability and adequacy of support, however, are not sufficient in themselves to assure a productive return on the Federal investment. Flexibility on the part of both the investigator and the funding agency is an additional critical factor. Flexibility manifests itself in serendipity, when the investigator recognizes the unexpected and seizes the opportunity to pursue an observation. For the granting agency, flexibility implies an understanding that planning can take an investigator only to a given point in research, that most

formal planning is soon out of date, and that the productive investigator requires flexibility to follow innovative leads.

Clinical research represents a special problem. Clinical studies are often complex, lengthy, and difficult to control. Nevertheless, statistically sound, adequately blinded studies following approved protocols are key factors in completing the transfer of technological advances. Every effort must be made to bring clinical investigations expeditiously to definitive conclusions to assure the public that it will benefit from safe and effective therapy. Such studies are costly, but the importance of getting definitive answers must be recognized. It is largely in the area of clinical applications that much of the Division's efforts at prevention are focused.

Almost everyone concerned with this report has also stressed the need for adequate data collection, appropriate registries, the support of specialized facilities and instrumentation, involvement in educational efforts directed at both lay and professional audiences, and the training of emerging scientists.

Methodology

This report was developed to serve several purposes:

- To provide the administration, the Congress, and the U.S. public with an accounting of what has been accomplished between 1972 and 1982 and with a prospectus for the future, on the occasion of the tenth anniversary of the National Heart, Blood Vessel, Lung, and Blood Act.

- To provide to the Director of the NHLBI the information needed for the Annual Report of the Director, as required by law.

- To provide the Division of Blood Diseases and Resources with a comprehensive assessment of progress over the last decade and of the current state of knowledge.

- To develop and refine the goals and objectives of the Division and to identify specific actions needed to maintain and accelerate advances in research and improvements in health.

This report was initiated in the spring of 1981 with the decision by the Director of the NHLBI that progress of the last 10 years be reviewed and that plans for the next 5 years be developed under the guidance of the three Divisions that support extramural

research programs of the NHLBI: the Division of Heart and Vascular Diseases, the Division of Lung Diseases, and the Division of Blood Diseases and Resources.

With the advice of the Blood Diseases and Resources Advisory Committee, an outline for the report, a procedure for its development, and a strategy for its preparation were developed by the DBDR during the summer of 1981. Members of the advisory committee agreed to chair working groups that would draft three sections of the report: Thrombosis and Hemostasis, Red Blood Cells and Their Disorders, and Blood Resources. Three additional sections were to be drafted by the staff of the Division: Magnitude of the Problem, Prevention, and Research Training and Development. Finally, an overview panel composed of the chairman of the Blood Diseases and Resources Advisory Committee, the principal hematologist for a project of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) to assess the state of the art in hematology, and the director of the DBDR was assigned the task of preparing this executive summary.

In the autumn of 1981, the key participants from the extramural community and from the Division met to review and refine the outline, revise the schedule, select additional contributors, and plan first drafts for their respective sections. In all, more than 50 experts worked jointly with the staff of the Division in the preparation of this report.

Beginning in the winter and continuing through the spring of 1982, initial drafts were received by the Division. The drafts were reviewed and modified by the Division staff, the working groups, and others. In May 1982, a final draft of each section was reviewed by the advisory committee. Based on their comments, this final report was prepared by the Division.

Resources include the following publications, to which the text makes frequent reference:

The National Heart, Blood Vessel, Lung, and Blood Program. DHEW Publ. Nos. (NIH) 73-515; 73-516; 73-517; 73-518; 73-519; 73-520; 73-521; 73-522; 73-523; and 73-524.

Annual reports of the Director, NHLI (later NHLBI), 1972 through 1981. DHEW Publ. Nos. (NIH) 74-514; 75-748; 76-970; 77-1170; 78-1415; 79-1605; 80-1672; 82-2103; and 84-2335.

Annual reports of the National Heart and Lung (later Heart, Lung, and Blood) Advisory Council, 1972 through 1981. DHEW Publ. Nos. (NIH) 74-508; 75-747; 76-971; 77-1171; 78-1418; 79-1606; 80-1673; 80-2104; and 81-2334.

First Report to the Director, DBDR, of the Blood Diseases and Resources Advisory Committee, 1976. DHEW Publ. No. (NIH) 76-1174.

Report of the NIADDK Committee to Review Hematology (not yet released).

Special mention should be made of the latter publication. Those responsible for the NIADDK report graciously allowed draft copies to be used by the Blood Diseases and Resources Advisory Committee. Thus, duplication of effort in assessing the current state of the science was avoided and up-to-date information was often readily available. To the chairman of that committee and to its members, the Blood Diseases and Resources Advisory Committee extends its thanks.

Many additional documents, reviews, and articles also served as resource materials. Where appropriate, they are identified within the report.

2. Magnitude of the Problem

Contents

2. Magnitude of the Problem

This section of the report of the Division of Blood Diseases and Resources addresses two fundamental questions: To what extent have blood diseases adversely affected the quality of life in the United States in recent years, and to what extent have blood resources improved the quality of life during the same period? To answer these questions, the status of blood diseases is analyzed for the period for which the most recent, relatively complete data are available (1978 and 1979); and changes in morbidity and mortality that occurred in the last decade (1970 through 1978) are discussed. The majority of the data are from the following studies, published and unpublished, of the National Center for Health Statistics (NCHS):

Type of Data	Source
Death statistics	Vital Statistics, NCHS
Hospital stays and days	Hospital Discharge Survey, NCHS
Physicians' office visits	Ambulatory Medical Care Survey, NCHS
Direct costs of health care	Health Care Financing Review, Health Care Financing Administration
Indirect costs of health care (morbidity and mortality costs)	National Center for Health Statistics

The data were analyzed and interpreted by the staff of the Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute. The data on blood resources are from two DBDR-sponsored surveys of blood collection, processing, and transfusion in the United States.

Blood Diseases

In 1979, the estimated economic cost of blood diseases* in the United States was $26.7 billion, of which approximately $7.7 billion were direct costs for hospital care, services of physicians and other health professionals, nursing home care, and drugs and medical sundries; and approximately $19 billion were indirect costs, including $2.9 billion for the cost of lost productivity as a result of illness (morbidity costs) and $16.1 billion as a result of premature deaths (mortality costs). In the same year, there were approximately 6.7 million visits by patients to physicians' offices for blood diseases. In 1978, death certificates listed blood diseases as a contributing factor 579,846 times, and blood diseases were reported as underlying causes** for 351,322 deaths. Blood diseases contributed to an estimated 3.3 million hospitalizations and were reported as primary reasons for approximately 1.4 million of these hospitalizations, which resulted in 16.1 million days of hospital care. Further details are presented in table 2, parts A and B.

Clotting Disorders

The clotting disorders include thromboembolic cerebrovascular and coronary artery diseases, phlebitis and thrombophlebitis, pulmonary embolism, and other venous and arterial thrombosis and embolism.

In 1978, these disorders contributed to more than 500,000 deaths and were reported as underlying causes of 345,482 deaths. Mortality statistics for specific clotting disorders are presented in table 3. These disorders contributed to approximately 2.2 million hospital stays and were reported as primary diagnoses in 1.1 million of these stays, which resulted in 13.9 million days of hospital care. In 1979, they were the reasons for approximately

*Blood diseases here refers both to the conditions traditionally considered blood diseases and to some patients with such diseases as myocardial infarction ("heart attack"), stroke, and pulmonary embolism, in which blood clotting plays a crucial role.

**Underlying cause is defined by the National Center for Health Statistics as "(a) the disease or injury which initiated the train of events leading directly to death, or (b) the circumstances of the accident or violence which produced the fatal injury."

Table 2. Magnitude of the Problem--Blood Diseases
Part A. Morbidity and Mortality

Indicators	Clotting Disorders	Bleeding Disorders	Sickle Cell Disease	Red Blood Cell Disorders	All Blood Diseases
Times reported as factors contributing to death (1978). This includes:	539,316	10,591	448	29,491	579,846
Times reported as primary cause of death	345,482	2,079	294	3,467	351,322
Number of hospitalizations contributed to (1978). This includes:	2,179,000	93,000	46,000	956,000	3,274,000
Number of hospitalizations caused by (1978). These resulted in:	1,075,000	29,000	27,000	247,000	1,378,000
Number of days of hospital care (1978)	13,892,000	224,000	180,000	1,834,000	16,130,000
Number of physicians' office visits (1979)	3,820,000	93,000	57,000	2,725,000	6,695,000

Table 2. Magnitude of the Problem--Blood Diseases
Part B. Economic Cost, 1979

Indicators	Clotting Disorders	Bleeding Disorders	Sickle Cell Disease	Red Blood Cell Disorders	All Blood Diseases
Direct costs for health care (Expenditures for hospitalization, professional services, nursing home care,* and drugs and medical sundries)	$ 6,539,000,000	$ 80,000,000	$59,000,000	$1,020,000,000	$ 7,698,000,000
Morbidity costs* (Economic value of productivity lost because of illness)	2,657,000,000	16,000,000	12,000,000	208,000,000	2,893,000,000
Mortality costs* (Economic value of productivity lost because of death)	15,775,000,000	24,000,000	18,000,000	311,000,000	16,128,000,000
TOTAL ECONOMIC COSTS	$24,971,000,000	$120,000,000	$89,000,000	$1,539,000,000	$26,719,000,000

*Nursing home, morbidity, and mortality costs for bleeding disorders, sickle cell disease, and red blood cell disorders are allocated on this table proportionally to the total of other direct health care costs for each of these three groups of disorders. All other costs are based on data that can be found on pages 90-101.

Table 3. Number of Deaths Involving Clotting Disorders, 1978

Diagnosis	Percent of Deaths Caused by Clotting	(ICDA 8) (Code)	Caused by Clotting Disorders	Contributed to by Clotting Disorders	Total Deaths Involving Clotting Disorders
Cerebral thrombosis	100	(433)	37,710	18,021	55,911
Cerebral embolism	100	(434)	760	2,595	3,355
Occlusion of precerebral arteries	80	(432)	1,851	1,211	3,062
Transient cerebral ischemia	80	(435)	66	341	407
Acute but ill-defined cerebrovascular disease	80	(436)	54,420	66,218	120,638
Generalized ischemic cerebrovascular disease	80	(437)	25,922	32,620	58,542
Other and ill-defined cerebrovascular disease	80	(438)	3,166	9,645	12,811
Acute myocardial infarction	68	(410)	205,812	16,577	222,389
Arterial embolism and thrombosis	100	(444)	1,916	12,652	14,568
Pulmonary embolism and infarction	100	(450)	10,941	28,279	39,220
Phlebitis and thrombophlebitis	100	(451)	1,740	2,503	4,243
Portal vein thrombosis	100	(452)	41	177	218
Other venous embolism and thrombosis	100	(453)	1,088	2,794	3,882
Puerperal phlebitis and thrombophlebitis	100	(671)	3	3	6
Puerperal pulmonary embolism	100	(673)	46	18	64
All clotting disorders			345,482	193,834*	539,316*

*These two totals may be greater than the actual number of deaths involving clotting disorders because of the recording of more than one clotting disorder on death certificates. All other numbers on this table are mutually exclusive.

Source: Published and unpublished vital statistics data from the National Center for Health Statistics.

3.8 million visits to physicians' offices (table 2, part A). In the same year, the total economic costs of clotting disorders were approximately $25 billion (table 2, part B). This amount consisted of:

$ 4,043,000,000	-- expenditures for hospital care
$ 404,000,000	-- expenditures for services of physicians and other health professionals
$ 1,868,000,000	-- expenditures for nursing home care
$ 224,000,000	-- expenditures for drugs and medical sundries
$ 2,657,000,000	-- lost productivity resulting from illness (morbidity cost)
$15,775,000,000	-- lost productivity resulting from death

Bleeding Disorders

The bleeding disorders include the genetic coagulation diseases (hemophilia A and B and von Willebrand's disease) and acquired hemorrhagic disorders.

These disorders contributed to about 10,000 deaths in 1978 and were reported as underlying causes of 2,079 of these deaths (table 2, part A). Mortality statistics for specific bleeding disorders are presented in table 4. Although the number of deaths is relatively small, more than 30 percent of deaths caused by the bleeding disorders occurred in persons under age 15.

In 1978, the bleeding disorders contributed to 93,000 hospital stays and were reported as primary diagnoses in 29,000 of these hospitalizations, which resulted in 224,000 days of hospital care. In 1979, there were 93,000 visits to physicians' offices for bleeding disorders (table 2, part A). In the same year, the estimated economic cost for bleeding disorders was $120 million (table 2, part B). Standard methods for calculating this sum probably underestimate the economic impact of bleeding disorders. About $40 million (or $4,000 per patient) was spent for antihemophilic factor by hemophilic patients. (The standard method of allocation for calculating cost estimates for all blood diseases results in $4 million for all drug expenditures for bleeding disorders.)

Table 4. Number of Deaths Involving Bleeding Disorders, 1978

	(ICDA 8) (Code)	Caused by Bleeding Disorders	Contributed to by Bleeding Disorders	Total Deaths Involving Bleeding Disorders
Hemophilia A	(286.0)	39	51	90
Hemophilia B	(286.1)	2	10	12
Other coagulation defects	(286.2-.9)	948	3,107	4,055
Thrombocytopenia	(287.1)	470	2,589	3,059
Other purpura and hemorrhagic conditions	(287.0, (287.2-.9)	122	592	714
Puerperal dyscrasia	(675)	9	9	18
Hemorrhagic diseases of the newborn	(778.2)	489	2,154	2,643
TOTAL		2,079	8,512*	10,591*

*These two totals may be greater than the actual number of deaths involving bleeding disorders because of the recording of more than one bleeding disorder on death certificates. All other numbers are mutually exclusive.

Source: Published and unpublished vital statistics data from the National Center for Health Statistics.

Sickle Cell Disease

The estimated prevalence of sickle cell anemia in the United States is 40,000 to 50,000 patients. The incidence of sickle cell anemia in births to black couples is about 1 in 540.

As reported on death certificates, sickle cell disease contributed to 448 deaths in 1978 and was the underlying cause for 294 of these deaths (table 2, part A). Two-thirds of the patients who died were under the age of 35. It is likely that the number of deaths of persons with sickle cell disease is larger than reported.

Sickle cell disease contributed to 46,000 hospital stays in 1978 and was reported as the primary diagnosis in 27,000 of these stays, which resulted in 180,000 days of hospital care. In 1979, there were 57,000 visits for sickle cell disease to physicians' offices (table 2, part A). In the same year, the estimated economic impact of sickle cell disease was $93 million (table 2, part B).

Red Blood Cell Disorders

The red blood cell disorders include deficiency anemias, the thalassemias, hereditary hemolytic anemias, and aplastic anemia.

In 1978, these disorders (excluding sickle cell disease) contributed to more than 25,000 deaths and were reported as primary causes of 3,467 deaths. The thalassemias (including Cooley's anemia) contributed to 103 deaths and were reported as underlying causes for 30 deaths; two-thirds of the deaths from the thalassemias occurred in persons under age 35. Mortality statistics for specific red blood cell disorders are presented in table 5.

The red blood cell disorders (excluding sickle cell disease) contributed to 956,000 hospital stays in 1978. They were diagnosed as primary causes in 247,000 of these stays, which resulted in approximately 1.8 million days of hospital care. In 1979, they were the reasons for approximately 2.7 million visits to physicians' offices. In the same year, the estimated economic impact of red blood cell disorders was $1.5 billion (table 2).

Table 5. Number of Deaths Involving Red Blood Cell Disorders, 1978

Diagnosis	(ICDA 8) (Code)	Caused by Red Blood Cell Disorders	Contributed to by Red Blood Cell Disorders	Total Deaths Involving Red Blood Cell Disorders
Disorders of iron and porphyrin metabolism	(273.1,273.2)	115	120	235
Iron deficiency anemias	(280)	111	1,379	1,490
Other deficiency anemias	(281)	286	1,868	2,154
Thalassemias	(282.4)	30	73	103
Hemoglobinopathies (principally sickle cell disease and its genetic variants)	(282.5)	294	154	448
Other hereditary hemolytic anemias	(282.0-.3, 282.9)	241	462	703
Acquired hemolytic anemias	(283)	139	196	335
Aplastic anemia	(284)	1,167	1,826	2,993
Other and unspecified anemias	(285)	1,032	18,217	19,249
Other diseases of blood and blood-forming organs	(289)	346	1,883	2,229
TOTAL		3,761	26,178*	29,939*

*These two totals may be greater than the actual number of deaths involving red blood cell disorders because of the recording of more than one red blood cell disorder on death certificates. All other numbers are mutually exclusive.

Source: Published and unpublished vital statistics data from the National Center for Health Statistics.

Patterns and Trends

The Bleeding and Clotting Disorders

The hemostatic system is the part of the body's defense mechanisms responsible for maintaining the blood in an optimal fluid state. Failures of maintenance of the blood in such a state within the blood vessels cause the bleeding and clotting disorders. Excessive bleeding can result from an abnormality or deficiency, acquired or inherited, of any of the processes in the hemostatic system. Bleeding into organs and tissues can impair or destroy their function, and massive, external blood loss can result in death.

Clotting in the arteries deprives a part of the body of its blood supply, and clotting in the veins impedes the return of blood to the heart. When excessive clotting affects the vasculature, the body's tissues and cells are deprived of essential oxygen, energy, regulating hormones, and building blocks; and noxious waste products cannot be effectively removed. The consequences of abnormal clotting depend on the organ or region of the body in which it occurs. In vessels of the heart, brain, lungs, or other vital organs, the result can be catastrophic; in less vital areas, the effects can be mild and transitory. With time, the clot-dissolving mechanism can restore the integrity of the circulation. The speed of this process, however, is insufficient in vital organs where even a very brief deprivation of blood can result in irreversible loss of function or death of tissue.

The impact of the clotting and bleeding disorders is considerable. Arterial thrombosis causes or complicates a variety of diseases in all parts of the body. Thrombosis involving the vessels of the kidney, for instance, contributes to kidney failure, and arterial thrombosis has a major role in stroke and heart attack. Thrombosis in the veins can produce pulmonary embolism--that is, clot fragments are carried in the blood from their site of origin to the lungs. Clotting in the venous circulation complicates many illnesses and surgical procedures.

The microcirculation conveys to the cells of the body the substances they need for their metabolism and regulation, and it removes their waste products. The process allows the cells to survive and to perform their interrelated tasks. Bleeding and clotting in the microcirculation are contributory or primary mechanisms in many disorders such as hypertension, stroke, diabetes, infectious and inflammatory diseases, autoimmune disease, host-graft rejection, cancer, sickle cell anemia, mismatched blood transfusion, liver disease, and nephritis. Excessive blood loss

due to inadequate or disturbed function of a part of the hemostatic mechanism is a major cause of death and morbidity in circumstances such as leukemia and cirrhosis of the liver and after severe injury. The threat of hemorrhage imposes limitations on the management of some forms of cancer.

The hemophilias, which are major bleeding disorders, are sex-linked, hereditary diseases that occur exclusively in males. Although the number of persons with moderate and severe hemophilia in this country is only 10,000 to 12,000, the disease constitutes a major national health problem. The cost of treatment, which must be continued throughout the lifetime of the patient, is enormous. In addition, treatment of this disease makes one of the largest single demands upon the nation's blood resources.

Mortality. Data for deaths associated with bleeding and clotting disorders are presented in tables 6 and 7. The numbers of deaths in the United States from 1970 through 1978 in which bleeding disorders were reported as causal or contributing factors were:

	1970	1972	1974	1976	1978
Bleeding disorders as:					
- underlying causes	1,487	1,646	1,996	2,113	2,079
- contributing factors					8,512

The numbers of deaths caused by each type of bleeding disorder are shown in table 6. The total number of deaths increased by 50 percent, from 1,487 in 1970 to 2,079 in 1978. This increase occurred in coagulation defects other than hemophilia. The numbers of deaths by age group were:

Age Group	1970	1972	1974	1976	1978
Under 1 year	558	568	716	739	589
1 through 14	52	38	46	51	57
15 through 64	472	500	602	611	604
65 and older	405	540	632	712	829
Total	1,487	1,646	1,996	2,113	2,079

Table 6. Number of Deaths Caused by Bleeding Disorders, 1970 to 1978*

Diagnosis	(ICDA 8) (Code)	Number of Deaths				
		1970	1972	1974	1976	1978
Hemophilia A	(286.0)	45	44	37	28	39
Christmas disease (Hemophilia B)	(286.1)	8	6	7	2	2
Other coagulation defects	(286.2-.9)	355	566	753	811	948
Thrombocytopenia	(287.1)	452	448	459	488	470
Other purpura and hemorrhagic conditions	(287.0, 287.2-.9)	124	122	144	132	122
Puerperal dyscrasias	(675)	11	12	8	8	9
Hemorrhagic disease of the newborn	(778.2)	492	448	588	644	489
TOTAL - All Bleeding Disorders		1,487	1,646	1,996	2,113	2,079

*Deaths for which these diagnoses were reported on death certificates as the underlying cause.
Source: National Center for Health Statistics.

Table 7. Number of Deaths Caused by Clotting Disorders, 1970 to 1978*

Diagnosis	Percent of Deaths Caused by Clotting	(ICDA 8) (Code)	Number of Deaths				
			1970	1972	1974	1976	1978
Cerebral thrombosis	100	(433)	57,845	57,482	53,542	44,803	37,710
Cerebral embolism	100	(434)	884	976	910	826	760
Occlusion of precerebral arteries	80	(432)	2,546	2,466	2,578	2,262	1,851
Transient cerebral ischemia	80	(435)	44	48	59	46	66
Acute but ill-defined cerebrovascular disease	80	(436)	47,134	52,362	54,345	54,311	54,420
Generalized ischemic cerebrovascular disease	80	(437)	26,354	29,635	31,081	28,412	25,922
Other and ill-defined cerebrovascular disease	80	(438)	2,179	2,518	2,798	2,939	3,166
Acute myocardial infarction	68	(410)	242,934	243,334	227,253	217,244	205,812
Arterial embolism and thrombosis	100	(444)	1,918	1,846	1,853	1,888	1,916
Pulmonary embolism and infarction	100	(450)	10,297	11,618	11,734	11,513	10,941
Phlebitis and thrombophlebitis	100	(451)	2,702	2,512	2,461	2,163	1,740
Portal vein thrombosis	100	(452)	93	70	55	46	41
Other venous embolism and thrombosis	100	(453)	1,770	1,688	1,556	1,312	1,088
Puerperal phlebitis and thrombophlebitis	100	(671)	14	16	10	3	3
Puerperal pulmonary embolism	100	(673)	115	98	69	54	46
TOTAL - All Clotting Disorders			396,829	406,669	390,304	367,822	345,482

*Deaths for which these diagnoses were reported on death certificates as the underlying cause.
Source: National Center for Health Statistics.

Trends in mortality for bleeding disorders by age group from 1970 through 1978 were:

- Deaths at 65 years of age and older increased by 105 percent
- Deaths at ages 15 through 64 increased by 28 percent
- Deaths at ages 1 through 14 increased by 10 percent
- Deaths under age 1 increased by 6 percent.

More than 90 percent of all deaths under age 1 were caused by hemorrhagic disease of the newborn.

The numbers of deaths reported as caused by hemophilia A from 1970 through 1978 were:

Age Group	1970	1972	1974	1976	1978
Under 1 year	1	2	0	0	1
1 through 14	10	8	3	6	4
15 through 64	31	28	19	18	15
65 and over	3	6	15	4	19
Total	45	44	37	28	39

The number of deaths caused by hemophilia declined by 13 percent between 1970 and 1978. A more significant trend is that deaths among persons 64 years of age and younger declined by 52 percent, from 42 to 20.

The numbers of deaths in the United States for 1970 through 1978 in which clotting disorders were reported as causal or contributing factors were:

	1970	1972	1974	1976	1978
Clotting disorders as:					
- underlying causes	396,829	406,669	390,304	367,822	345,482
- contributing factors					193,834

The numbers of deaths caused by each type of clotting disorder are given in table 7. The data presented in the table for acute myocardial infarction* and some types of cerebrovascular disease** are far less than 100 percent of all deaths caused by these disorders.

Trends in mortality from 1970 through 1978 for clotting disorders, based on the data presented in table 7, were:

- The total number of deaths declined by 13 percent, from 396,829 to 345,482.
- The percent decline in deaths for individual clotting disorders for this period include 62 percent for puerperal clotting disorders, 39 percent for venous embolism and thrombosis, 36 percent for phlebitis and thrombophlebitis, and 35 percent for cerebral thrombosis and embolism.
- The largest decreases in numbers of deaths were 37,000 for acute myocardial infarction and 20,000 for cerebral thrombosis.

*It is possible, and some would assert probable, that thrombosis participates in the pathology of most coronary artery disease, which includes acute myocardial infarction, other acute coronary artery disease, and chronic coronary artery disease. The strongest current evidence for the participation of thrombosis in these disorders relates to acute myocardial infarction only. Clinical investigators have recently reported that treatment of such cases with a thrombolytic (clot-dissolving) agent early in the postinfarct period results in reestablishment of blood flow beyond the point of occlusion in more than two-thirds of acute myocardial infarct patients. Conservative estimates of the health and cost effects are used throughout this discussion, and therefore, only a percentage of acute myocardial infarctions is estimated to involve thrombosis. Estimates for other forms of coronary artery disease are not included.

**A recent report of a study supported by the National Institute of Neurological and Communicative Disorders and Stroke provides estimates showing that 85 to 90 percent of all cerebrovascular disease involves thrombosis or embolism. For this discussion, data for cerebral hemorrhage have been excluded; 100 percent of reported morbidity and mortality data for cerebral thrombosis and embolism and 80 percent of morbidity and mortality data for other types of cerebrovascular disease, however, have been included.

- The types of cerebrovascular diseases reported to have caused increasing numbers of deaths during this period were acute but ill-defined cerebrovascular disease, which increased by 15 percent, and other ill-defined cerebrovascular disease, which increased by 45 percent.

Morbidity. In quantitative terms, morbidity is the sum of the adverse effects (excluding death) of a disease on patients during a specified period of time (usually 1 year). The amount of morbidity that is caused by a disease or that is contributed to by a disease is not precisely determinable. Estimates can be made, however, with the use of such data as number and duration of hospitalizations, number of physicians' office visits, lost days of school or work, and days of restricted activity for patients with a disease.

From 1970 through 1978, the hospital stays to which bleeding disorders contributed increased by 60 percent (from 58,000 to 93,000) (table 8). Nearly all the increase resulted from a 158 percent increase in the hospital stays to which thrombocytopenia contributed (from 19,000 to 49,000) (table 9). Although the number of hospital stays to which these diagnoses contributed increased by 60 percent, the number of hospital stays for which bleeding disorders were the primary (first-listed) diagnosis increased by only 26 percent (from 23,000 to 29,000) (table 8). The entire increase resulted from increased hospitalizations for thrombocytopenia (table 10). During the same period, the total number of days of hospitalization (which ranged from 192,000 in 1970 to 272,000 in 1975) and the average number of days of hospitalization per admission (ranging from 6.9 in 1977 to 9.7 in 1975) showed no significant trend when bleeding disorders were the primary (first-listed) diagnoses (tables 8 and 11).

During the same period, the hospital stays to which clotting disorders contributed increased by 38 percent (from 1.6 million to 2.2 million) (table 12). With the exception of hospital stays to which ill-defined cerebrovascular disease contributed (a decrease of almost 50 percent), all other groups of clotting disorders contributed to the 38 percent increase (table 13). The largest percentage increase (136 percent) was in stays to which occlusion of the precerebral arteries contributed. The largest numeric increase (125,000) was in stays to which acute myocardial infarcts contributed (table 13). The number of hospital stays for which clotting disorders were the primary (first-listed) diagnoses increased by 30 percent (from 825,000 to 1,075,000) (table 12). Almost all groups of clotting disorders contributed to this increase (table 13). The total number of days of hospitalization for stays for which clotting disorders were the primary (first-listed) diagnoses increased by 16 percent (from 11.9 million to

Table 8. Hospitalization Data for All Bleeding Disorders, 1970 to 1978

	A	B	C	D = C÷B	E = B÷A
	Total Hospital Stays to Which Bleeding Disorders Contributed	Total Hospital Stays for Which Bleeding Disorders Were the Primary (First-Listed) Diagnosis	Total Days of Hospitalization for Which Bleeding Disorders Were the Primary (First-Listed) Diagnosis	Average Length of Hospital Stay in Days When Bleeding Disorders Were the Primary (First-Listed) Diagnosis	Percent of Hospital Stays to Which Bleeding Disorders Contributed as the Primary (First-Listed) Diagnosis
1970	58,000	23,000	192,000	8.3	(40)
1971	49,000	27,000	229,000	8.5	(55)
1972	63,000	28,000	223,000	8.0	(44)
1973	60,000	27,000	235,000	8.7	(45)
1974	62,000	26,000	212,000	8.2	(42)
1975	74,000	28,000	272,000	9.7	(38)
1976	79,000	27,000	215,000	8.0	(34)
1977	83,000	29,000	200,000	6.9	(35)
1978	93,000	29,000	224,000	7.7	(31)

BLEEDING DISORDERS

First-Listed Diagnosis

Hospital Discharges (in thousands)

Diagnosis	(ICDA-8) (Code)	1970	1971	1972	1973	1974	1975	1976	1977	1978
Hemophilia A	(286.0)	7	7	8	6	5	6	5	4	5
Hemophilia B (Christmas disease)	(286.1)	1	1	2	1	*	1	1	1	1
Other coagulation defects	(286.2-9)	4	4	5	4	5	3	5	4	5
Thrombocytopenia	(287.1)	7	9	7	10	8	10	10	12	13
Other purpura and hemorrhagic conditions	(287.0,.2-.9)	4	6	6	6	8	8	6	8	5
Puerperal dyscrasias	(675)	*	*	*	*	*	*	*	*	*
Hemorrhagic disease of the newborn	(778.2)	*	*	*	*	*	*	*	*	*
TOTAL - All Bleeding Disorders		23	27	28	27	26	28	27	29	29

*Represents fewer than 1,000 discharges.

Table 9. Hospital Discharges--Bleeding Disorders, 1970 to 1978
First-Listed Diagnosis

Table 10. Hospital Discharges--Bleeding Disorders, 1970 to 1978
All Listed Diagnoses

BLEEDING DISORDERS

All Listed Diagnoses

Diagnosis	(ICDA-8) (Code)	Hospital Discharges (in thousands) 1970	1971	1972	1973	1974	1975	1976	1977	1978
Hemophilia A	(286.0)	8	8	10	9	6	7	6	6	7
Hemophilia B (Christmas disease)	(286.1)	1	1	4	1	1	1	2	2	2
Other coagulation defects	(286.2-.9)	13	12	15	15	17	18	22	24	23
Thrombocytopenia	(287.1)	19	18	20	23	24	31	37	38	49
Other purpura and hemorrhagic conditions	(287.0,.2-.9)	11	8	13	11	13	17	12	12	11
Puerperal dyscrasias	(675)	1	1	1	*	*	*	*	1	*
Hemorrhagic disease of the newborn	(778.2)	5	1	*	1	1	*	*	*	1
TOTAL - All Bleeding Disorders		58	49	63	60	62	74	79	83	93

*Represents fewer than 1,000 discharges.

Table 11. Days of Hospital Care--Bleeding Disorders, 1970 to 1978

BLEEDING DISORDERS

First-Listed Diagnosis

Diagnosis	(ICDA-8) (Code)	Days of Hospital Care (in thousands)								
		1970	1971	1972	1973	1974	1975	1976	1977	1978
Hemophilia A	(286.0)	31	37	66	36	37	31	16	24	35
Hemophilia B (Christmas disease)	(286.1)	2	2	8	6	1	2	7	3	6
Other coagulation defects	(268.2-.9)	25	51	34	22	32	54	23	23	32
Thrombocytopenia	(287.1)	86	107	69	115	90	113	127	106	128
Other purpura and hemorrhagic conditions	(287.0,.2-.9)	48	32	42	55	52	72	42	44	22
Puerperal dyscrasias	(675)	*	*	4	1	*	*	*	*	*
Hemorrhagic disease of the newborn	(778.2)	*	*	*	*	*	*	*	*	1
TOTAL - All Bleeding Disorders		192	229	223	235	212	272	215	200	224

*Represents fewer than 1,000 discharges.

Table 12. Hospitalization Data for All Clotting Disorders, 1970 to 1978

	A	B	C	D = C÷B	E = B÷A
	Total Hospital Stays to Which Clotting Disorders Contributed	Total Hospital Stays for Which Clotting Disorders Were the Primary (First-Listed) Diagnosis	Total Days of Hospitalization for Which Clotting Disorders Were the Primary (First-Listed) Diagnosis	Average Length of Hospital Stay in Days When Clotting Disorders Were the Primary (First-Listed) Diagnosis	Percent of Hospital Stays to Which Clotting Disorders Contributed as the Primary (First-Listed) Diagnosis
1970	1,583,000	825,000	11,927,000	14.5	(52)
1971	1,565,000	833,000	11,995,000	14.4	(53)
1972	1,783,000	926,000	12,870,000	13.9	(52)
1973	1,918,000	956,000	13,232,000	13.8	(50)
1974	2,028,000	1,021,000	13,621,000	13.3	(50)
1975	2,114,000	1,039,000	13,760,000	13.2	(49)
1976	2,132,000	1,064,000	14,156,000	13.3	(50)
1977	2,218,000	1,109,000	14,074,000	12.7	(50)
1978	2,179,000	1,075,000	13,892,000	12.9	(49)

Table 13. Hospital Discharges--Clotting Disorders, 1970 to 1978
First-Listed Diagnosis

CLOTTING DISORDERS

First-Listed Diagnosis — Hospital Discharges (in thousands)

Diagnosis	(ICDA 8) (Code)	1970	1971	1972	1973	1974	1975	1976	1977	1978
Phlebitis and thrombophlebitis of venous sinuses (100%)	(321)	*	*	1	*	1	*	*	*	*
Cerebral thrombosis (100%)	(433)	88	82	96	95	95	85	88	91	90
Cerebral embolism (100%)	(434)	4	6	5	9	9	6	9	9	8
Occlusion of precerebral arteries (80%)	(432)	23	22	24	31	34	40	40	50	53
Transient cerebral ischemia (80%)	(435)	19	22	26	31	39	37	35	38	40
Acute but ill-defined cerebrovascular disease (80%)	(436)	158	160	172	173	183	186	202	206	210
Generalized ischemic cerebrovascular disease (80%)	(437)	84	86	99	102	96	95	92	82	80
Other and ill-defined cerebrovascular disease (80%)	(438)	22	21	21	19	25	21	23	20	16
Acute myocardial infarction (68%)	(410)	233	228	254	241	260	265	272	281	289
Arterial embolism and thrombosis (100%)	(444)	25	30	29	37	35	36	36	44	40
Pulmonary embolism and infarction (100%)	(450)	48	51	62	73	83	84	87	92	73
Phlebitis and thrombophlebitis (100%)	(451)	109	115	124	133	149	169	164	173	155
Portal vein thrombosis (100%)	(452)	*	*	*	*	*	*	*	*	*
Other venous embolism and thrombosis (100%)	(453)	12	9	12	12	12	14	15	23	19
Puerperal phlebitis and thrombophlebitis (100%)	(671)	*	1	1	*	*	1	1	*	1
Puerperal pulmonary embolism (100%)	(673)	*	*	*	*	*	*	*	*	1
TOTAL - All Clotting Disorders		825	833	926	956	1,021	1,039	1,064	1,109	1,075

*Represents fewer than 1,000 discharges.

13.8 million days). The average length of stay per hospitalization, however, decreased by 11 percent (from 14.5 to 12.9 days) (table 12). A major contributor to this trend was the length of stay for acute myocardial infarct, which decreased from 16.3 to 12.5 days per stay (tables 14 and 15).

All measures of hospitalization for arterial thrombosis and embolism increased from 1970 through 1978. Total hospital stays increased by 59 percent (from 70,000 to 111,000); hospital stays for which these disorders were the primary diagnosis increased by 60 percent (from 25,000 to 40,000); total days of hospitalization increased by 101 percent (from 321,000 to 645,000); and the average length of stay per hospitalization increased by 26 percent (from 12.8 to 16.1 days). Ninety percent of hospital stays and days of care was for patients 45 years of age and older (table 16).

Except for a relatively stable average length of stay per hospitalization (about 14 days), from 1970 through 1978 every measure of hospitalization for pulmonary embolism increased. Total hospital stays increased by 58 percent (from 113,000 to 179,000); hospital stays for which pulmonary embolism was the primary diagnosis increased by 52 percent (from 48,000 to 73,000); and total days of hospitalization increased by 46 percent (from 680,000 to 996,000). Eighty percent of hospital stays and days of care was for patients 45 years of age and older (table 17).

Except for an average length of stay of 11 days per hospitalization, from 1970 through 1978 every other measure of hospitalization for phlebitis and thrombophlebitis increased. Total hospital stays increased by 45 percent (from 174,000 to 252,000); and hospital stays for which these disorders were the primary diagnoses increased by 42 percent (from 109,000 to 155,000). Seventy percent of hospital stays and days of care was for patients 45 years of age and older (table 18).

Sickle Cell Disease

Sickle cell disease encompasses sickle cell anemia and its genetic variants. In persons with sickle cell anemia, red blood cells become deformed and rigid under certain conditions, particularly when deoxygenated. The deformed cells tend to block the small capillaries of the circulatory system and prevent oxygen from reaching tissues. Chronic organ damage can result.

Hemoglobin S is the most common of all abnormal hemoglobins in the United States. About 8.6 percent of U.S. blacks carry the sickle cell gene--that is, they have sickle cell trait. In equatorial Africa, as many as half the members of certain tribes

Table 14. Hospital Discharges--Clotting Disorders, 1970 to 1978
All Listed Diagnoses

CLOTTING DISORDERS

All Listed Diagnoses

Hospital Discharges (in thousands)

Diagnosis	(ICDA 8) (Code)	1970	1971	1972	1973	1974	1975	1976	1977	1978
Phlebitis and thrombophlebitis of venous sinuses (100%)	(321)	*	*	1	1	1	*	1	*	*
Cerebral thrombosis (100%)	(433)	144	133	157	161	158	152	156	160	157
Cerebral embolism (100%)	(434)	16	16	15	23	24	23	24	21	24
Occlusion of precerebral arteries (80%)	(432)	45	42	50	63	73	89	91	103	106
Transient cerebral ischemia (80%)	(435)	32	34	48	54	66	66	66	69	72
Acute but ill-defined cerebrovascular disease (80%)	(436)	238	232	249	276	294	311	325	354	363
Generalized ischemic cerebrovascular disease (80%)	(437)	234	239	280	310	310	236	311	295	290
Other and ill-defined cerebrovascular disease (80%)	(438)	108	100	98	86	90	75	75	68	55
Acute myocardial infarction (68%)	(410)	384	379	435	432	464	478	491	503	525
Arterial embolism and thrombosis (100%)	(444)	70	71	87	93	96	103	97	116	111
Pulmonary embolism and infarction (100%)	(450)	113	116	139	179	183	197	198	213	179
Phlebitis and thrombophlebitis (100%)	(451)	174	175	195	212	239	263	263	273	252
Portal vein thrombosis (100%)	(452)	1	1	*	1	1	*	1	*	1
Other venous embolism and thrombosis (100%)	(453)	22	21	27	24	27	28	31	41	40
Puerperal phlebitis and thrombophlebitis (100%)	(671)	2	4	2	2	1	2	2	1	2
Puerperal pulmonary embolism (100%)	(673)	*	2	*	1	1	1	*	1	2
TOTAL - All Clotting Disorders		1,583	1,565	1,783	1,918	2,028	2,114	2,132	2,218	2,179

*Represents fewer than 1,000 discharges.

Table 15. Days of Hospital Care--Clotting Disorders, 1970 to 1978

CLOTTING DISORDERS

First-Listed Diagnosis

Diagnosis	(ICDA 8) (Code)	Days of Hospital Care (in thousands) 1970	1971	1972	1973	1974	1975	1976	1977	1978
Phlebitis and thrombophlebitis of venous sinuses (100%)	(321)	*	2	11	3	20	7	6	*	1
Cerebral thrombosis (100%)	(433)	1,525	1,416	1,596	1,578	1,540	1,431	1,452	1,389	1,484
Cerebral embolism (100%)	(434)	47	83	80	142	136	103	120	114	126
Occlusion of precerebral arteries (80%)	(432)	280	242	289	355	412	422	400	488	532
Transient cerebral ischemia (80%)	(435)	132	165	192	238	300	307	258	290	317
Acute but ill-defined cerebrovascular disease (80%)	(436)	2,514	2,490	2,441	2,688	2,774	2,818	2,944	2,902	3,280
Generalized ischemic cerebrovascular disease (80%)	(437)	993	1,066	1,122	1,108	1,114	976	960	898	829
Other and ill-defined cerebrovascular disease (80%)	(438)	260	210	290	235	319	270	306	287	221
Acute myocardial infarction (68%)	(410)	3,788	3,758	3,971	3,762	3,741	3,779	3,875	3,777	3,618
Arterial embolism and thrombosis (100%)	(444)	321	397	414	441	417	488	465	572	645
Pulmonary embolism and infarction (100%)	(450)	680	740	907	1,105	1,139	1,194	1,250	1,212	996
Phlebitis and thrombophlebitis (100%)	(451)	1,238	1,321	1,406	1,426	1,574	1,810	1,927	1,842	1,626
Portal vein thrombosis (100%)	(452)	2	*	*	6	*	1	3	1	3
Other venous embolism and thrombosis (100%)	(453)	141	100	148	141	133	144	184	302	204
Puerperal phlebitis and thrombophlebitis (100%)	(671)	4	5	3	3	1	7	6	*	5
Puerperal pulmonary embolism (100%)	(673)	2	0	0	1	1	3	*	*	5
TOTAL - All Clotting Disorders		11,927	11,995	12,870	13,232	13,621	13,760	14,156	14,074	13,892

*Represents fewer than 1,000 discharges.

Table 16. Hospital Discharges and Days of Care--Arterial Embolism and Thrombosis, 1970 to 1978

ARTERIAL EMBOLISM AND THROMBOSIS (444)	1970	1971	1972	1973	1974	1975	1976	1977	1978
First-Listed Diagnosis	Hospital Discharges (in thousands)								
- under 15 years of age	*	*	*	1	*	*	*	1	*
- 15 to 44 years of age	3	2	3	4	4	3	4	3	3
- 45 to 64 years of age	11	12	13	14	16	14	15	19	17
- 65 and more years of age	12	16	13	18	14	18	16	21	20
Total - All Ages	25	30	29	37	35	36	36	44	40
All Listed Diagnoses									
- under 15 years of age	1	1	*	1	*	1	1	2	1
- 15 to 44 years of age	7	6	9	10	9	10	10	10	9
- 45 to 64 years of age	28	31	36	34	41	39	40	47	44
- 65 and more years of age	33	34	42	48	45	53	47	57	57
Total - All Ages	70	71	87	93	96	103	97	116	111
First-Listed Diagnosis	Days of Hospital Care (in thousands)								
- under 15 years of age	4	3	*	5	*	1	2	4	7
- 15 to 44 years of age	19	38	31	50	33	34	46	34	43
- 45 to 64 years of age	130	151	172	180	204	177	220	200	291
- 65 and more years of age	168	205	211	206	180	276	197	334	304
Total - All Ages	321	397	414	441	417	488	465	572	645

*Represents fewer than 1,000 discharges or days.

Table 17. Hospital Discharges and Days of Care--Pulmonary Embolism, 1970 to 1978

PULMONARY EMBOLISM (450)	1970	1971	1972	1973	1974	1975	1976	1977	1978
First-Listed Diagnosis	Hospital Discharges (in thousands)								
- under 15 years of age	*	*	1	1	1	*	*	*	1
- 15 to 44 years of age	11	10	15	19	20	18	19	20	13
- 45 to 64 years of age	19	19	23	27	31	33	31	33	26
- 65 and more years of age	18	22	23	27	32	33	36	38	33
Total - All Ages	48	51	62	73	83	84	87	92	73
All Listed Diagnoses									
- under 15 years of age	*	*	1	1	1	1	1	*	1
- 15 to 44 years of age	21	21	25	34	36	35	37	38	29
- 45 to 64 years of age	44	43	52	63	64	77	74	75	61
- 65 and more years of age	48	52	62	81	83	84	87	100	89
Total - All Ages	113	116	139	179	183	197	198	213	179
First-Listed Diagnosis	Days of Hospital Care (in thousands)								
- under 15 years of age	*	*	4	8	1	5	5	*	9
- 15 to 44 years of age	139	140	175	240	259	209	233	225	140
- 45 to 64 years of age	249	253	353	417	419	475	463	434	342
- 65 and more years of age	295	347	375	439	460	505	550	554	505
Total - All Ages	680	740	907	1,105	1,139	1,194	1,250	1,212	996

*Represents fewer than 1,000 discharges or days.

Table 18. Hospital Discharges and Days of Care--Phlebitis and Thrombophlebitis, 1970 to 1978

PHLEBITIS AND THROMBOPHLEBITIS (451)	1970	1971	1972	1973	1974	1975	1976	1977	1978
First-Listed Diagnosis	Hospital Discharges (in thousands)								
- under 15 years of age	1	1	*	1	1	1	1	*	1
- 15 to 44 years of age	35	40	37	43	48	52	53	57	44
- 45 to 64 years of age	41	45	52	50	60	67	62	67	59
- 65 and more years of age	33	30	35	39	41	51	49	49	51
Total - All Ages	109	115	124	133	149	169	164	173	155
All Listed Diagnoses									
- under 15 years of age	1	1	*	2	1	1	1	1	2
- 15 to 44 years of age	50	51	52	62	68	76	77	83	62
- 45 to 64 years of age	66	70	79	83	94	105	102	102	96
- 65 and more years of age	57	53	63	65	76	82	84	87	92
Total - All Ages	174	175	195	212	239	263	263	273	252
First-Listed Diagnosis	Days of Hospital Care (in thousands)								
- under 15 years of age	6	6	*	7	3	6	5	3	8
- 15 to 44 years of age	373	391	397	418	459	487	573	540	402
- 45 to 64 years of age	472	507	571	539	645	716	725	689	643
- 65 and more years of age	387	417	438	462	467	601	625	610	573
Total - All Ages	1,238	1,321	1,406	1,426	1,574	1,810	1,927	1,842	1,626

*Represents fewer than 1,000 discharges or days.

have sickle cell trait. Hemoglobin S is also found in nonblack populations. Incidence of 25 to 30 percent has been reported in populations in southern Turkey, Saudi Arabia, southern India, and in villages in Greece, Sicily, and Cyprus. There are not sufficient data, however, on which to base estimates of worldwide incidence and prevalence of sickle cell trait and anemia.

In the United States, the numbers of deaths of patients with sickle cell anemia and its variants reported from 1970 through 1978 were:

	1970	1972	1974	1976	1978
Sickle cell anemia as:					
- the underlying cause	390	394	307	304	294
- a contributing factor					154
Total					448

Of the deaths for which sickle cell anemia and its genetic variants were the reported underlying cause, the distribution by age was:

Age Group	1970	1972	1974	1976	1978
Under 1 year	15	30	9	20	10
1 through 14 years	109	92	82	59	68
15 through 64 years	257	260	209	214	203
65 years and over	9	12	7	11	13
Total	390	394	307	304	294

Both the total number of deaths and the number of deaths in ages 1 through 14 showed a downward trend during the 1970's.

Data on hospitalization provide primary indicators of patterns and trends in morbidity associated with sickle cell disease. From 1970 through 1978, the total number of hospital stays to which sickle cell anemia and its genetic variants contributed increased by 77 percent (from 26,000 to 46,000); the hospital stays for which sickle cell anemia and its genetic variants were the primary (first-listed) diagnosis increased by 80 percent (from 15,000 to 27,000); the number of days of hospitalization increased by 33 percent (from 135,000 to 180,000); and the average length of stay per hospitalization decreased by 26 percent (from 9.0 to 6.7 days). From 1970 through 1978, more than 90 percent of hospital stays and days was by patients 44 years of age and younger, and most of the increase in hospital days and stays was for patients 15 through 44 years of age (table 19).

Table 19. Hospital Discharges and Days of Care--Sickle Cell Disease, 1970 to 1978

SICKLE CELL DISEASE AND ITS GENETIC VARIANTS (282.5)	1970	1971	1972	1973	1974	1975	1976	1977	1978
First-Listed Diagnosis	Hospital Discharges (in thousands)								
- under 15 years of age	6	10	6	7	6	8	8	5	8
- 15 to 44 years of age	8	7	10	15	13	14	17	18	18
- 45 to 64 years of age	1	1	2	1	1	1	1	2	1
- 65 and more years of age	*	1	1	*	*	1	1	*	*
Total - All Ages	15	19	19	23	20	24	27	25	27
All Listed Diagnoses									
- under 15 years of age	9	12	11	12	12	12	12	11	11
- 15 to 44 years of age	15	15	18	23	23	28	27	28	31
- 45 to 64 years of age	2	2	4	3	3	4	5	5	3
- 65 and more years of age	*	1	*	1	*	2	2	1	1
Total - All Ages	26	30	33	39	40	46	46	46	46
First-Listed Diagnosis	Days of Hospital Care (in thousands)								
- under 15 years of age	53	61	39	42	37	36	39	30	61
- 15 to 44 years of age	67	81	91	110	89	112	146	155	112
- 45 to 64 years of age	14	17	7	8	4	7	8	14	7
- 65 and more years of age	1	1	1	2	4	5	*	1	*
Total - All Ages	135	160	138	162	134	160	193	200	180

*Represents fewer than 1,000 discharges or days.

Red Blood Cell Disorders

There are hundreds of red blood cell disorders. Some are acquired; others are congenital. They result from one of four general causes occurring singly or in combination: production of too few red cells, production of impaired red cells, accelerated destruction of red cells, and loss of blood. Red blood cell disorders may be the most frequently occurring group of chronic diseases.

Among the acquired disorders, iron deficiency anemia is estimated to occur in most developed countries in approximately 3 percent of male adults, 20 percent of female adults, and more than 20 percent of children. Where data are available, the rates are considerably higher in undeveloped countries.

Among the congenital disorders, about 200 million people are affected by a genetic red cell deficiency, glucose-6-phosphate dehydrogenase deficiency (G-6-PD). The prevalence of sickle cell hemoglobin has already been noted. The thalassemias are another frequently occurring group of genetic red blood cell disorders, which are common in the Mediterranean basin (southern Europe and northern Africa) and extend through the Middle East and India to the Orient. Tens of millions of persons carry a thalassemia gene, but the worldwide prevalence of thalassemia is not known. The most severe thalassemia found in the United States is Cooley's anemia. It occurs in fewer than 1,000 Americans, largely those of Mediterranean ancestry.

Most of the red blood cell disorders are not incompatible with life. They are chronic disorders that are often left untreated or are treated in outpatient settings. Death and hospitalization statistics consequently do not give a true picture of the total effect of these disorders on U.S. health.

The numbers of deaths in the United States from 1970 through 1978 in which red blood cell disorders were reported as causal or contributing factors were:

	1970	1972	1974	1976	1978
Red blood cell disorders as:					
- underlying causes	3,859	3,872	3,742	3,612	3,761
- contributing factors					26,024

The numbers of deaths from 1970 through 1978 caused by each type of disorder are given in table 20. The total number decreased by

Table 20. Number of Deaths Caused by Red Blood Cell Disorders, 1970 to 1978*

Diagnosis	(ICDA 8) (Code)	Number of Deaths				
		1970	1972	1974	1976	1978
Disorders of iron and porphyrin metabolism	(273.1, 273.2)	118	122	131	125	115
Iron deficiency anemias	(280)	96	78	86	90	111
Other deficiency anemias	(281)	509	394	340	253	286
Thalassemias	(282.4)	30	30	30	40	30
Hemoglobinopathies (principally sickle cell anemia and its genetic variants)	(282.5)	390	394	307	304	294
Other hereditary hemolytic anemias	(282.0-.3, 282.9)	250	220	235	215	241
Acquired hemolytic anemias	(283)	105	124	139	90	139
Aplastic anemia	(284)	1,055	1,168	1,089	1,085	1,167
Other and unspecified anemias	(285)	992	1,018	1,086	1,105	1,032
Other diseases of blood and blood-forming organs	(289)	314	324	299	305	346
TOTAL - Red Blood Cell Disorders		3,859	3,872	3,742	3,612	3,761

*Deaths for which these diagnoses were the underlying cause reported on death certificates.

Source: National Center for Health Statistics.

3 percent (from 3,859 to 3,761). Most of this decrease was in deaths caused by deficiency anemias and by sickle cell disease.

The numbers of deaths caused by red blood cell disorders reported in the United States from 1970 through 1978, by age group, were:

Age Group	1970	1972	1974	1976	1978
Under 1 year	76	94	47	62	41
1 through 14	241	242	219	154	176
15 through 64	1,247	1,292	1,139	1,025	1,013
65 and older	2,295	2,244	2,337	2,371	2,531
Total	3,859	3,872	3,742	3,612	3,761

During this period, the mortality trends by age group are 46 percent decrease at under 1 year of age, 27 percent decrease from age 1 through 14, 19 percent decrease from age 15 through 64, and 10 percent increase at 65 years and older. In 1970, 8 percent of deaths occurred in persons 14 years of age and younger; and in 1978, only 6 percent of deaths occurred in this age group. In 1970, 59 percent of deaths occurred in persons 65 years of age and older; and by 1978, deaths in this age group increased to 67 percent. The numbers of deaths reported as caused by the thalassemias (including Cooley's anemia) and the distribution of ages at death have remained relatively constant, between 27 and 40 per year. About 60 percent of these deaths occurred in patients 15 through 64 years of age.

Major observations about red blood cell disorders can be based on hospitalization data for 1970 through 1978. The total number of hospital stays to which red blood cell disorders contributed increased by 44 percent (from 697,000 to 1,002,000); the hospital stays for which red blood cell disorders were the primary (first-listed) diagnosis increased by 22 percent (from 225,000 to 274,000); the total days of hospitalization averaged about 2 million days per year; and the average length of stay per hospitalization decreased by 13 percent (from 8.5 to 7.4 days) (table 21). By 1978, red blood cell disorders were the primary diagnosis in only one of four hospital stays to which they contributed; in 1970, they were the primary diagnosis in one of three stays (table 21). All groups of blood disorders contributed to these trends (tables 22 through 24). As with sickle cell anemia, patients hospitalized for the thalassemias (including Cooley's anemia) were primarily in the younger groups; more than 90 percent of the patients with a primary diagnosis of thalassemia were 44 years of age or younger and accounted for 80 percent of days of hospitalization (table 25).

Table 21. Hospitalization Data for All Red Blood Cell Disorders, 1970 to 1978

	A Total Hospital Stays to Which Red Blood Cell Disorders Contributed	B Total Hospital Stays for Which Red Blood Disorders Were the Primary (First-Listed) Diagnosis	C Total Days of Hospitalization for Which Red Blood Cell Disorders Were the Primary (First-Listed) Diagnosis	D = C÷B Average Length of Hospital Stay in Days When Red Blood Disorders Were the Primary (First-Listed) Diagnosis	E = B÷A Percent of Hospital Stays to Which Red Blood Cell Disorders Contributed as the Primary (First-Listed) Diagnosis
1970	697,000	225,000	1,902,000	8.5	(32)
1971	725,000	221,000	1,813,000	8.2	(30)
1972	855,000	231,000	1,872,000	8.1	(27)
1973	867,000	245,000	1,994,000	8.1	(28)
1974	935,000	249,000	2,054,000	8.2	(27)
1975	1,042,000	267,000	2,123,000	8.0	(26)
1976	1,031,000	273,000	2,140,000	7.8	(26)
1977	987,000	269,000	1,969,000	7.3	(27)
1978	1,002,000	274,000	2,014,000	7.4	(27)

Table 22. Hospital Discharges--Red Blood Cell Disorders, 1970 to 1978
All Listed Diagnoses

RED BLOOD CELL DISORDERS

All Listed Diagnoses

Diagnosis	(ICDA 8) (Code)	Hospital Discharges (in thousands) 1970	1971	1972	1973	1974	1975	1976	1977	1978
Disorders of iron and porphyrin metabolism	(273.1-.2)	3	3	5	6	6	4	4	7	6
Iron deficiency anemias	(280)	232	233	281	281	303	347	325	302	299
Other deficiency anemias	(281)	60	59	66	65	70	72	71	66	64
Thalassemias	(282.4)	5	4	5	6	7	10	15	15	13
Hemoglobinopathies (principally sickle cell disease and its genetic variants)	(282.5)	26	30	33	39	40	46	46	45	46
Other hereditary hemolytic anemias	(282.0-.3, 282.9)	9	9	12	12	12	15	17	15	14
Acquired hemolytic anemias	(283)	3	3	2	6	5	5	5	5	5
Aplastic anemia	(284)	18	15	18	22	21	26	27	30	31
Other and unspecified anemias	(285)	221	237	253	286	316	370	369	352	363
Other diseases of blood and blood-forming organs	(289)	120	132	130	144	155	147	150	150	161
TOTAL - Red Blood Cell Disorders		697	725	855	867	935	1,042	1,031	987	1,002

Table 23. Hospital Discharges--Red Blood Cell Disorders, 1970 to 1978
First-Listed Diagnosis

RED BLOOD CELL DISORDERS

First-Listed Diagnosis

Diagnosis	(ICDA 8) (Code)	Hospital Discharges (in thousands) 1970	1971	1972	1973	1974	1975	1976	1977	1978
Disorders of iron and porphyrin metabolism	(273.1-.2)	1	1	2	2	2	3	3	4	4
Iron deficiency anemias	(280)	53	51	57	55	59	67	62	65	62
Other deficiency anemias	(281)	22	21	21	19	18	18	16	14	13
Thalassemias	(282.4)	3	2	2	2	2	5	6	7	5
Hemoglobinopathies (principally sickle cell disease and its genetic variants)	(282.5)	15	19	19	23	20	24	27	25	27
Other hereditary hemolytic anemias	(282.0-.3, 282.9)	4	4	4	4	6	5	6	7	5
Acquired hemolytic anemias	(283)	1	1	1	3	2	2	2	3	3
Aplastic anemia	(284)	12	9	12	12	9	13	12	14	14
Other and unspecified anemias	(285)	44	43	42	47	51	56	65	54	66
Other diseases of blood and blood-forming organs	(289)	70	70	71	78	80	74	74	76	75
TOTAL - Red Blood Cell Disorders		225	221	231	245	249	267	273	269	274

Table 24. Days of Hospital Care--Red Blood Cell Disorders, 1970 to 1978

RED BLOOD CELL DISORDERS

Diagnosis	(ICDA 8) (Code)	Days of Hospital Care (in thousands)								
		1970	1971	1972	1973	1974	1975	1976	1977	1978
Disorders of iron and porphyrin metabolism	(273.1-.2)	13	11	20	16	16	12	39	37	35
Iron deficiency anemias	(280)	516	460	522	497	574	620	554	566	574
Other deficiency anemias	(281)	301	252	250	238	218	227	218	152	144
Thalassemias	(282.4)	11	11	16	5	16	33	19	23	11
Hemoglobinopathies (principally sickle cell disease and its genetic variants)	(282.5)	135	160	138	162	134	160	193	200	180
Other hereditary hemolytic anemias	(282.0-.3, 282.9)	44	32	33	38	69	60	53	71	39
Acquired hemolytic anemias	(283)	15	14	27	42	21	23	48	32	33
Aplastic anemia	(284)	117	71	109	99	77	128	82	111	117
Other and unspecified anemias	(285)	410	441	389	503	459	496	577	434	501
Other diseases of blood and blood-forming organs	(289)	340	361	368	394	380	364	357	343	380
TOTAL - Red Blood Cell Disorders		1,902	1,813	1,872	1,994	2,054	2,123	2,140	1,969	2,014

THALASSEMIAS (282.4)	1970	1971	1972	1973	1974	1975	1976	1977	1978
First-Listed Diagnosis	Hospital Discharges (in thousands)								
- under 15 years of age	1	1	1	1	1	3	4	4	4
- 15 to 44 years of age	1	1	1	1	1	1	2	2	1
- 45 to 64 years of age	1	*	*	*	*	1	*	1	*
- 65 and more years of age	*	*	*	*	*	*	*	*	*
Total - All Ages	3	2	2	2	2	5	6	7	5
All Listed Diagnoses									
- under 15 years of age	2	1	1	1	2	3	5	5	5
- 15 to 44 years of age	2	2	2	3	4	4	6	6	4
- 45 to 64 years of age	*	1	2	1	1	2	2	2	2
- 65 and more years of age	1	*	*	1	*	1	2	2	2
Total - All Ages	5	4	5	6	7	10	15	15	13
First-Listed Diagnosis	Days of Hospital Care (in thousands)								
- under 15 years of age	2	1	3	1	5	5	6	5	5
- 15 to 44 years of age	4	8	5	4	10	21	9	13	6
- 45 to 64 years of age	*	2	7	*	1	3	4	2	*
- 65 and more years of age	5	*	1	*	*	4	1	3	*
Total - All Ages	11	11	16	5	16	33	19	23	11

*Represents fewer than 1,000 discharges or days.

Blood Resources

Each year, blood resources are used in the treatment of millions of patients in the United States who suffer from hundreds of different diseases. The object of all transfusion therapy is to restore the patient to normal physiologic status. Numerous products are prepared from blood and its components or as substitutes for blood components for transfusion to meet specific patient needs.

Quantities of Blood Collected

Blood can be collected (drawn) whole. In addition, selected components (plasma, white cells, or platelets) can be collected by the technique of apheresis. Apheresis is a process by which freshly drawn blood is separated into its components, some of which are kept and the remainder of which are reinfused into the donor.

Most whole blood is drawn by community and regional blood centers. These same institutions also perform almost all plateletapheresis and leukapheresis procedures. The vast majority of plasmapheresis procedures, however, are performed by the pharmaceutical industry. Such plasma, together with some that is purchased from blood centers and hospitals, is used in the preparation of blood products such as antihemophilic factor, albumin, and immune globulins.

Data on collection are uneven. Data on units of whole blood collected in 1971 and 1979 in the United States are available from studies supported by the DBDR. Data on units of platelets and white cells (leukocytes) collected by apheresis, which are also from a DBDR-sponsored study, are available for 1979 only. Estimates of liters* of source plasma** collected by the pharmaceutical industry have appeared in recent issues of Plasma Quarterly, the journal of the American Blood Resources Association.

*On the average, 10 liters of plasma are collected from 15 to 16 plasmapheresis procedures.

**Source plasma is defined by the Food and Drug Administration as "the fluid portion of human blood collected by plasmapheresis and intended as source material for further manufacturing use."

The data from the DBDR-supported studies of collection by regional and community blood centers and hospitals are:

	1971	1979
Units of whole blood	8,799,700	10,822,867
- by blood centers	6,038,700	9,552,877
- by hospitals	1,797,000	1,070,409
- by others	964,000	63,873
- from paid donors	964,000	490,964
Units of leukocytes (One apheresis procedure provides one unit of leukocytes)		38,198
Units of platelets (One apheresis procedure provides eight units of platelets)		653,616

These data show that, from 1971 to 1979, collection of whole blood increased by 23 percent, that regional and community blood centers increased their collection by about 3.5 million units and their share of collection from 69 to 88 percent of all units, that hospital collection declined by 720,000 units and their share of collection from 20 to 10 percent, and that units of blood collected from paid donors decreased by almost 475,000 units from 11 to 4.5 percent of all units of blood collected.

The estimates of liters of source plasma collected are:

1971	1976	1978	1979
1,550,000	2,340,000	3,087,500	4,210,000

These estimates indicate that source plasma collected by apheresis increased by 2.7 million liters, or by 172 percent, from 1971 to 1979. Between 5 million and 6 million Americans donated whole blood in 1979. Another 250,000 to 300,000 persons gave plasma, and an additional 25,000 to 50,000 persons gave white cells and platelets by apheresis.

Quantities of Blood Products

The best available data come from the results of the DBDR-supported survey in 1979 of blood centers and hospitals. All other data are based on either expert opinion or unverified

reports, or are statistical projections based on data that may be nonrepresentative.

The 1979 survey showed that the following products were prepared from approximately 10.9 million units of whole blood collected in that year:

- 7,271,073 units of red cells
- 3,070,085 units of platelets
- 2,049,551 units of plasma for transfusion
- 537,683 units of cryoprecipitated antihemophilic factor
- 3,551,794 units kept as whole blood (Because this number includes an unknown quantity lost through breakage and screening, it is a theoretical maximum.)

Hospitals and blood centers prepared approximately 16.5 million units of transfusible products, and approximately 1.4 million liters of plasma were recovered for use by the pharmaceutical industry in the manufacture of plasma products. Of the whole blood collected, 67 percent was used for component preparation. A total of 16.5 million units of transfusible products was processed from whole blood, or 1.52 units of transfusible products per unit of whole blood. This 1979 datum is comparable to 1.15 units in 1971, based on data published by the American Red Cross.

Component therapy, which provides each patient with only the component(s) needed, is recognized as the most effective medical approach to patient care. Because an increase in the production of therapeutic units from each unit of whole blood expands the limited supply of resources, this trend is positive.

Estimates for the quantity of selected products manufactured by the pharmaceutical industry from source plasma and recovered plasma have appeared in recent editions of Plasma Quarterly. These estimates are:

	1971	1976	1979
Units of antihemophilic factor (AHF)	80,000,000	400,000,000	687,500,000
Units of albumin (12.5-gram units)	3,100,000	5,300,000	7,300,000

Quantities of Blood Products Transfused

The availability and quality of data vary by transfused product. Accurate estimates for units of whole blood, red cells,

platelets, plasma, and cryoprecipitated antihemophilic factor transfused in the United States in 1971 and 1979 were compiled in the two DBDR studies, which show that nearly all transfusions (99.8 percent) of whole blood, red cells, platelets, plasma, and cryoprecipitate are performed in hospitals and that the rest are performed by blood centers. In 1971 and 1979, the following numbers of units, in total and for each of these five blood products, were transfused:

	1971	1979
Total units transfused	7,368,300	13,389,615
- as whole blood	4,972,400	2,165,321
- as red cells	1,394,100	7,306,449
- as platelets	413,500	2,219,573
- as plasma	183,300	1,285,686
- as cryoprecipitated antihemophilic factor	405,000	412,586

These data show that the total number of units of the five products transfused increased by more than 6 million units from 1971 to 1979, or by 82 percent. The units of whole blood transfused decreased by 2.8 million, or by 56 percent. The units of cryoprecipitated antihemophilic factor transfused were unchanged. The units of the other three blood products increased enormously: red cells by almost 6 million units, or 424 percent; platelets by 1.8 million units, or 437 percent; and plasma by 1.1 million units, or 601 percent.

Estimates of the number of 12.5-gram units of albumin and units of antihemophilic factor produced for domestic (U.S.) consumption have appeared in Plasma Quarterly:

	1971	1976	1979
Albumin (12.5-gram units)	2,800,000	4,600,000	5,800,000
Antihemophilic factor (AHF) units	72,000,000	300,000,000	412,500,000

There are no data, however, for the number of units transfused in the United States.

Patients Transfused With Whole Blood and Red Cells

The only accurate estimates for patients transfused with whole blood and red cells are from 1979 data in the two DBDR studies. In 1979, about 2.9 million patients received 9.5 million units of whole blood and red cells. Since there were 36.2 million hospital discharges in 1979, about 7.9 percent of all hospitalized patients received transfusions of whole blood or red cells. The average (mean) number of units transfused per patient was 3.3 units. The number of patients transfused and the number of units transfused are shown in table 26 by major disease category.

Adverse Effects

Recipients of whole blood, its components, derivatives, and substitutes occasionally experience adverse transfusion reactions, and transfusion-transmitted hepatitis is the most frequent serious complication of blood transfusion. As a result of the universal practice in the United States of screening donor blood for type B hepatitis virus and of the decline in use of commercial blood, the incidence of hepatitis B resulting from transfusion has been reduced by between 80 and 90 percent. Recent reports indicate that only 10 to 13 percent of cases of transfusion-transmitted hepatitis are now due to type B virus infection. The remainder is type non-A,non-B, for which no screening tests are available.

The incidence of transfusion-transmitted hepatitis in patients who have received whole blood or red cells is at least 7 percent and possibly as high as 10 percent. Most recent reports have shown type non-A,non-B hepatitis incidence at about 7 percent. With the assumption of an additional 1 percent for type B hepatitis, it is likely that about 8 percent of patients who receive whole blood or red cells are infected by hepatitis virus.

In 1979, approximately 2.8 million persons were transfused with whole blood or red cells. On the assumption that 7 percent were infected with non-A,non-B hepatitis virus and that 1 percent were infected with type B virus, the incidence of transfusion-transmitted hepatitis in 1979 in recipients of whole blood and red cells was:

Non-A,non-B hepatitis	200,200
Type B hepatitis	28,600
Total cases of transfusion-transmitted hepatitis	228,800

In addition to whole blood and red cells, all blood products except albumin can transmit hepatitis. Most important among these

Table 26. Estimated Whole Blood and Red Cell Transfusion in 1979

Major Disease Category	Units		Patients	
	Number of Units	Percent of. Units	Number of Patients	Percent of Transfused Patients
All categories	9,471,000*	100.0	2,860,000*	100.0
1. Malignant neoplasms	1,771,000***	18.7**	523,000***	18.3**
2. Cardiovascular and cerebrovascular	1,525,000	16.1	386,000	13.5
3. Gastrointestinal	1,487,000	15.7	400,000	14.0
4. Trauma	1,136,000	12.0	340,000	11.9
5. Blood disorders	492,000	5.2	146,000	5.1
6. Obstetrical procedures and complications	407,000	4.3	172,000	6.0
7. Bone and joint	331,000	3.5	117,000	4.1
8. Respiratory	265,000	2.8	86,000	3.0
9. Liver	256,000	2.7	54,000	1.9
10. Kidney and bladder	256,000	2.7	86,000	3.0
11. Gynecologic and breast	246,000	2.6	112,000	3.9
12. Complications	152,000	1.6	49,000	1.7
13. Perinatal	142,000	1.5	46,000	1.6

*Total number of units transfused and patients transfused from the National Blood Data Center.
**Percentages of transfusion by diagnosis from " A Study of National Trends in Transfusion Practices" (Friedman, et al.).
***Estimates by the NHLBI of units and patients for all major disease categories.

Table 26. Estimated Whole Blood and Red Cell Transfusion in 1979 (continued)

Major Disease Category	Units		Patients	
	Number of Units	Percent of Units	Number of Patients	Percent of Transfused Patients
14. Benign tumors	142,000	1.5	54,000	1.9
15. Gallbladder	114,000	1.2	40,000	1.4
16. Male genital	114,000	1.2	43,000	1.5
17. Metabolic	95,000	1.0	31,000	1.1
18. Skin and soft tissue	76,000	0.8	26,000	0.9
19. Infection	76,000	0.8	20,000	0.7
20. Hernias	66,000	0.7	20,000	0.7
21. Diabetes complications	57,000	0.6	20,000	0.7
22. Pancreas	38,000	0.4	11,000	0.4
23. Nervous system	38,000	0.4	14,000	0.5
24. Fluid blance	19,000	0.2	9,000	0.3
25. Endocrine	19,000	0.2	6,000	0.2
26. Eye and ear	9,000	0.1	3,000	0.1
27. Miscellaneous	142,000	1.5	46,000	1.6
TOTAL	9,471,000		2,860,000	

products is antihemophilic factor (AHF), which is made from plasma pooled from many donors. This pooling is necessary to produce at a reasonable cost AHF in needed amounts. The pooling, however, increases the probability that a unit of AHF will transmit hepatitis. Because each of the 10,000 to 12,000 persons with severe hemophilia use tens of thousands of units of AHF each year, all these hemophiliacs are believed to have hepatitis.

Economic Cost

The estimated economic cost of blood diseases in 1979 was $26.7 billion (table 27). This sum represents the effects of blood diseases on the nation's economy. It is composed of two types of costs: direct costs, including hospital care, the services of physicians and other health professionals, nursing home care, and drugs and medical sundries; and indirect costs, including lost productivity* resulting from illness (morbidity costs) and from death (mortality costs). Estimates of morbidity and mortality costs are reasonably complete. Some direct costs, however, cannot be allocated to blood diseases. Such costs are for construction of medical facilities, government public health expenditures, research, and other health services and administration.

Direct Costs

The Health Care Financing Administration (HCFA) has estimated that the direct costs for patient care in the United States in 1979 were $165.4 billion. This total consists of:

$85,342,000,000	- Expenditures for patient care at all U.S. hospitals
$45,286,000,000	- Expenditures for services of physicians and other health-related professionals

*Lost productivity includes the costs of lost earnings and the value of lost homemaking services. Morbidity costs result from earnings lost in 1979; mortality costs include, for those who died in 1979, earnings lost in 1979 and lost potential future earnings.

Table 27. Economic Cost of Blood Diseases, United States, 1979
(in millions of dollars)

Diagnosis	Hospital Care	Physicians' and Other Health Professional Services	Nursing Home Care	Drugs and Drug Sundries	Total Direct Costs	Morbidity Costs	Mortality Costs	Total Costs
Bleeding Disorders	$ 56	$ 11	NA*	$ 4	$ 71**	NA	NA	$ 71†
Sickle Cell Disease	44	6	NA	3	53**	NA	NA	53†
Red Blood Cell Disorders	498	300	NA	112	910**	NA	NA	910†
All Bleeding and Red Cell Disorders	$598	$317	$125	$119	$1,159	$236	$353	$1,748
Cerebrovascular Disease	$1,994	$170	$ 944	$ 96	$3,204	$ 901	$ 4,993	$ 9,098
Coronary Artery Disease	1,063	91	62	50	1,266	408	10,586	12,260
Arterial Thrombosis and Embolism	190	15	740	8	953	77	50	1,080
Phlebitis and Thrombophlebitis	437	102	68	56	663	699	38	1,400
Pulmonary Embolism	291	13	44	7	355	465	84	904
Venous Embolism and Thrombosis	68	13	10	7	98	107	24	229
All Clotting Disorders	$4,043	$404	$1,868	$224	$6,539	$2,657	$15,775	$24,971
ALL BLOOD DISEASES	$4,641	$721	$1,993	$343	$7,698	$2,893	$16,128	$26,719

*NA - not available.
**Excludes nursing home costs for which no technically acceptable basis for allocation was available.
†Excludes nursing home, morbidity, and mortality costs for which no technically acceptable basis for allocation was available.
Note: See tables 27 through 36 for sources of data and calculations used to develop these estimates of the economic costs of blood diseases.

$17,807,000,000	- Expenditures for nursing home care
$16,975,000,000	- Expenditures for drugs and medical sundries

In a Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975," it was estimated that 0.7 percent of the expenditures for health care was for diseases of the blood and blood-forming organs. Included in this category were red blood cell disorders and bleeding disorders. Multiplying the $85.3 billion for hospital care by 0.7 percent gives $598 million for hospital care for the red cell and bleeding disorders. In table 28, this amount has been allocated to the three groups of diseases of the blood and blood-forming organs (bleeding disorders, sickle cell disease, and red blood cell disorders excluding sickle cell disease). The amount is based on the proportion of all hospital days that each group contributed to total hospital days. A similar method has been used to estimate expenditures for services of physicians and other health professionals (table 29), nursing home care (table 30), and drugs and medical sundries (table 31).

Excluded from this section of the report are expenditures for malignant diseases and diseases of the immune system, which are outside the mission of the NHLBI. Clotting (thromboembolic) disorders, however, are a major responsibility of the DBDR, and the direct estimated costs for these disorders are shown in tables 32 through 35.

Because some vascular diseases are outside the responsibility of the Division of Blood Diseases and Resources, the direct costs shown in tables 32 through 35 are a subset of the direct costs reported by the Division of Heart and Vascular Diseases (DHVD). The method used to compute the costs of clotting disorders differs from that used to compute the costs of the red cell disorders and bleeding disorders. For each major diagnostic category (cerebrovascular disease, coronary artery disease, arterial thrombosis and embolism, phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis), the DHVD's data for each type of expenditure in 1979 were used as the baseline. Within each diagnostic category, the proportion of direct costs attributable to clotting disorders was calculated by the DBDR using data provided by the National Center for Health Statistics. Arterial thrombosis and embolism, for example, is included in the DHVD's diagnostic group titled "Diseases of Other Arteries." As shown in table 32, there were 3.5 million days of hospital care in 1979 for "diseases of other arteries" at a cost of $1 billion. Data from the Hospital Discharge Survey of the NCHS, which show that 645,000 (18.2 percent) of the 3.5 million days of hospital care were for arterial thrombosis and embolism, have provided the basis for

Table 28. Direct Costs--Expenditures for Hospital Care for Bleeding and Red Cell Disorders, 1979

Diagnosis	Hospital Days* (in thousands)	Percent Distribution	Expenditures (in millions)
Bleeding Disorders	224	9.3	$ 56
Sickle Cell Disease	180	7.4	44
Other Red Blood Cell Disorders	2,014	83.3	498
TOTAL - All Red Blood Cell and Bleeding Disorders	2,418	100.0	$598**

*Unpublished data from the Hospital Discharge Survey of the National Center for Health Statistics.
**Estimated by applying 0.7%, as determined in the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975," to the $85,342,000,000 total health expenditures for hospital care in 1979 as reported by the HCFA.

Table 29. Direct Costs--Expenditures for Services of Physicians and Other Health Professionals for Bleeding and Red Blood Cell Disorders, 1979

Diagnosis	Physicians' Office Visits* (in thousands)	Percent Distribution	Expenditures (in millions)
Bleeding Disorders	93	3.4	$ 11
Sickle Cell Disease	57	2.1	6
Other Red Blood Cell Disorders	2,610	94.5	300
TOTAL - All Red Blood Cell and Bleeding Disorders	2,760	100.0	$317**

*Unpublished data from the Ambulatory Medical Care Survey of the NCHS.
**Estimated by applying 0.7%, as determined in the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975," to the $45,286,000,000 total health expenditures for services of physicians and other health professionals in 1979 as reported by the HCFA.

Table 30. Direct Costs--Expenditures for Nursing Home Care for Bleeding and Red Blood Cell Disorders, 1979

Diagnosis	Days of Care (in thousands)	Percent Distribution	Expenditures (in millions)
Bleeding Disorders	NA*	NA	NA
Sickle Cell Disease	NA	NA	NA
Other Red Blood Cell Disorders	NA	NA	NA
TOTAL - All Red Blood Cell and Bleeding Disorders	NA	100.0	$125**

*NA - not available.
**Estimated by applying 0.7%, as determined in the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975," to the $17,807,000,000 total health expenditures for nursing home care in 1979 as reported by the HCFA.

Table 31. Direct Costs--Expenditures for Drugs and Medical Sundries for Bleeding and Red Blood Cell Disorders, 1979

Diagnosis	Physicians' Office Visits* (in thousands)	Percent Distribution	Expenditures (in millions)
Bleeding Disorders	93	3.4	$ 4
Sickle Cell Disease	57	2.1	3
Other Red Blood Cell Disorders	2,610	94.5	112
TOTAL - All Red Blood Cell and Bleeding Disorders	2,760	100.0	$119**

*Unpublished data from the Ambulatory Medical Care Survey of the NCHS.
**Estimated by applying 0.7%, as determined in the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975," to the $16,975,000,000 total health expenditures for drugs and medical sundries in 1979 as reported by the HCFA.

Table 32. Direct Costs--Expenditures for Hospital Care for Clotting Disorders, 1979

Diagnosis	Total Hospital Days (in thousands)	Hospital Days for Clotting Disorders	Percent of Total Days for Clotting Disorders	Hospital Expenditures (in millions of dollars)	
				Total	For Clotting Disorders
Cerebrovascular Disease	9,225	6,789	73.6	$2,709	$1,994
Coronary Artery Disease	16,695	3,618	21.7	4,905	1,063
Arterial Thrombosis and Embolism*	3,543	645	18.2	1,041	190
Phlebitis and Thrombophlebitis**	7,139	1,489	20.9	2,096	437
Pulmonary Embolism**		990	13.9		291
Venous Embolism and Thrombosis**		232	3.2		68
TOTAL - All Clotting Disorders		13,892			$4,043

*Hospital costs and days of stay for arterial thrombosis and embolism are included in DHVD's diagnostic group titled "Diseases of Other Arteries" (arteriosclerotic peripheral vascular diseases).

**Hospital costs and days of stay for phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis are included in DHVD's diagnostic group "other," which includes nonarteriosclerotic peripheral vascular diseases.

Note: These estimates are based on 1979 hospital cost data calculated by the DHVD using data from the NCHS and the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975." Numbers of hospital days for clotting disorders within each diagnosis are from unpublished data from the Hospital Discharge Survey of the NCHS.

Table 33. Direct Costs--Expenditures for Services of Physicians and Other Health Professionals for Clotting Disorders, 1979

Diagnosis	Total Physicians' Visits (in thousands)*	Visits for Clotting Disorders	Percent of Visits for Clotting Disorders	Expenditures for Professional Services (in millions of dollars)	
				Total	For Clotting Disorders
Cerebrovascular Disease	1,817	1,603	88.2	$193	$170
Coronary Artery Disease	9,133	871	9.5	949	91
Arterial Thrombosis and Embolism*	2,291	144	6.2	240	15
Phlebitis and Thrombophlebitis**	6,329	963	15.2	668	102
Pulmonary Embolism**		119	1.9		13
Venous Embolism and Thrombosis**		121	1.9		13
TOTAL - All Clotting Disorders		3,820			$404

*Visits to physicians' offices for arterial thrombosis and embolism are included in DHVD's diagnostic group titled "Diseases of Other Arteries" (arteriosclerotic peripheral vascular diseases).

**Visits to physicians' offices for phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis are included in DHVD's diagnostic group "other," which includes nonarteriosclerotic peripheral vascular diseases.

Note: These estimates are based on 1979 costs for professional services calculated by the DHVD using data from the NCHS and the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975." Numbers of physician visits for clotting disorders within each diagnosis are from unpublished data from the Ambulatory Medical Care Survey of the NCHS.

Table 34. Direct Costs--Expenditures for Nursing Home Care for Clotting Disorders, 1979

Diagnosis	Total Nursing Home Expenditures (in millions of dollars)	Estimated Percent for Clotting Disorders	Costs for Clotting Disorders (in millions of dollars)
Cerebrovascular Disorders	$1,282	73.6	$944
Coronary Artery Disease	286	21.7	62
Arterial Thrombosis and Embolism*	4,067	18.2	740
Phlebitis and Thrombophlebitis**	323	20.9	68
Pulmonary Embolism**		13.9	44
Venous Embolism and Thrombosis**		3.2	10
TOTAL - All Clotting Disorders			$1,868

*Costs for nursing home care for arterial thrombosis and embolism are included in DHVD's diagnostic group titled "Diseases of Other Arteries" (arteriosclerotic peripheral vascular diseases).

**Costs for nursing home care for phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis are included in DHVD's diagnostic group "other," which includes nonarteriosclerotic peripheral vascular diseases.

Note: These estimates are based on 1979 costs for nursing home care calculated by the DHVD. The percentages for costs for clotting disorders were estimated by the DBDR.

Table 35. Direct Costs--Expenditures for Drugs and Medical Sundries for Clotting Disorders, 1979

Diagnosis	Total Physicians' Visits (in thousands)*	Visits for Clotting Disorders	Percent of Visits for Clotting Disorders	Expenditures for Drugs and Sundries (in millions of dollars)	
				Total	For Clotting Disorders
Cerebrovascular Disease	1,817	1,603	88.2	$107	$ 96
Coronary Artery Disease	9,133	871	9.5	525	50
Arterial Thrombosis and Embolism*	2,291	144	6.2	133	8
Phlebitis and Thrombophlebitis**	6,329	963	15.2	369	56
Pulmonary Embolism**		119	1.9		7
Venous Embolism and Thrombosis**		121	1.9		7
TOTAL - All Clotting Disorders		3,912			$224

*Visits to physicians' offices for arterial thrombosis and embolism are included in DHVD's diagnostic group titled "Diseases of Other Arteries" (arteriosclerotic peripheral vascular diseases).

**Visits to physicians' offices for phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis are included in DHVD's diagnostic group "other," which includes nonarteriosclerotic peripheral vascular diseases.

Note: These estimates are based on 1979 costs for professional services calculated by the DHVD using data from the NCHS and the Georgetown University study, "Cost of Illness and Disease, Fiscal Year 1975." Numbers of physician visits for clotting disorders within each diagnosis are from unpublished data from the Ambulatory Medical Care Survey of the NCHS.

calculating that $190 million (18.2 percent of $1 billion) was expended in 1979 for hospital care for arterial thrombosis and embolism. A similar method was used to estimate the expenditures for each type of direct health care for each diagnostic category presented in tables 32 through 35.

Indirect Costs

Estimates of morbidity and mortality costs for bleeding and red blood cell disorders in 1977 were made by the NCHS. The estimates have been increased by a factor for inflation and are shown in table 36 for 1979. As with direct costs, estimates for morbidity and mortality costs for clotting disorders are a subset of the morbidity and mortality costs in selected diagnostic groups reported by the DHVD. In the calculation of the percent contribution of clotting disorders to these costs, estimates reported by the DHVD from NCHS data were used as the base. The results are presented in table 37.

Blood Resources

Table 38 presents estimated costs for selected blood resources and services in the United States in 1979. The expenditures for five types of blood products (whole blood and red cells, plasma, platelets, cryoprecipitate, and antihemophilic factor) and one blood resource service (therapeutic apheresis) totaled $912.8 million.

Table 36. Estimated Morbidity and Mortality Costs for Red Blood Cell Disorders, 1979

Diagnosis	Costs (in millions of dollars) Morbidity	Mortality
Bleeding Disorders	NA*	NA
Sickle Cell Disease	NA	NA
Other Red Blood Cell Disorders	NA	NA
TOTAL - All Red Blood Cell and Bleeding Disorders	$236**	$353**

*NA - not available.

**Estimates for 1979 are based on morbidity and mortality costs for Health Interview Survey recodes 099, 100, and 101 from the NCHS. Estimates for 1977 were used, and an inflation factor of 17.8% was applied.

Table 37. Estimated Morbidity and Mortality Costs for Clotting Disorders, 1979
(in millions of dollars)

Diagnosis	Total Morbidity Costs	Percent of Total Morbidity for Clotting Disorders	Morbidity Costs for Clotting Disorders	Total Mortality Costs	Percent of Total Mortality for Clotting Disorders	Mortality Costs for Clotting Disorders
Cerebrovascular Disease	$1,022	88.2	$ 901	$ 6,803	73.4	$ 4,993
Coronary Artery Disease	1,880	21.7	408	29,082	36.4	10,586
Arterial Thrombosis and Embolism*	497	15.5	77	1,110	4.5	50
Phlebitis and Thrombophlebitis**		17.7	699		1.0	38
Pulmonary Embolism**	3,939	11.8	465	3,836	2.2	84
Venous Embolism and Thrombosis**		2.7	107		0.6	24
TOTAL - All Clotting Disorders			$2,657			$15,775

*Arterial thrombosis and embolism are included in the DHVD's diagnostic group titled "Diseases of Other Arteries" (arteriosclerotic peripheral vascular diseases).

**Phlebitis and thrombophlebitis, pulmonary embolism, and venous embolism and thrombosis are included in the DHVD's diagnostic group "other," which includes nonarteriosclerotic peripheral vascular diseases.

Note: These estimates are based on 1979 morbidity and mortality costs calculated by the DHVD. The percentage of total costs for clotting disorders was developed by the DBDR using unpublished data from the NCHS; for example, the percentage of mortality for each diagnostic category was calculated by dividing the number of deaths caused by clotting disorders by the total number of deaths in that diagnostic category.

Table 38. Expenditures for Selected Blood Resources and Services, 1979

Product	Units Transfused	Average Charge Per Unit	Total
Whole blood and red cells	9,471,770	$79.00	$748,269,830
Plasma	1,285,686	30.00	38,570,580
Platelets	2,219,573	30.00	66,587,190
Cryoprecipitate	412,586	15.00	6,188,790
Antihemophilic factor	412,500,000	0.10	41,250,000

Service	Number of Procedures	Average Charge	Total
Therapeutic plasmapheresis	15,949	$750.00	$ 11,961,750
TOTAL for Selected Blood Resources and Services			$912,828,140

Note: No data were available upon which to base firm estimates of the expenditures for other blood products. These products include albumin, plasma protein fraction, and the immune globulins. Millions of units of these products are used in the United States annually.

3. Thrombosis and Hemostasis

Contents

3. Thrombosis and Hemostasis

This section of the report of the Division of Blood Diseases and Resources is divided into four content areas: normal hemostasis, platelet disorders, hemophilia and acquired bleeding disorders, and thromboembolism. This is also a planning document, and in instances where topics overlap substantially, material presented in detail in one passage may be summarized in another.

At least two crucial caveats should head a planning document in biomedical research:

- One cannot plan to discover what one does not know about. Landmark discoveries in biomedical science that open new research horizons often result from an astute investigator's insights into the significance of a chance observation that might be entirely unrelated to the original purpose of the research. The Nobel prize-winning discovery of the hepatitis B surface antigen, for example, resulted from an observation made in a study of plasma protein allotypes in Australian aborigines. It would have been impossible to "plan" to discover the hepatitis B surface antigen in plasma; nobody had the slightest idea it was there.

- Long-term plans based upon technical methods of a given moment often become obsolete before the planning period expires. Indeed, the rate of obsolescence is a useful measure of scientific progress. In the National Heart, Blood Vessel, Lung, and Blood Program of 1972, no mention was made of research based upon the two technical advances that have revolutionized scientific approaches of the past several years: the restriction endonucleases that have made recombinant DNA technologies possible, and monoclonal antibodies that permit molecular investigations of the structure and function of specific proteins.

Flexibility of goals and designs, permitting exploitation of chance observations, is a touchstone of basic research. In contrast, clinical research focuses upon specific practical diagnostic and therapeutic questions, and it may involve a different approach. Very costly both in time and effort of investigators and in the dollars they require, cooperative clinical studies should be initiated only after a careful assessment of the clinical importance of the questions that they are designed to answer,

of the adequacy of the knowledge upon which the designs are based, and of the merit of the proposed protocols.*

Normal Hemostasis

The processes that maintain the fluidity of blood within blood vessels are known collectively as hemostasis. When a blood vessel is injured, hemostatic reactions arrest bleeding through the action of its platelets and the blood coagulation factors. A seal made up of aggregated platelets with a scaffold of supporting strands of clotted fibrin is formed at the site of the injury. When platelets contact the tissues beneath the inner lining of the wall of the injured vessel, they become activated, and the resulting interrelated reactions cause them to stick to the tissues and then to each other. Simultaneously, a separate sequence of other delicately regulated reactions lead to the generation of the enzyme thrombin. Thrombin splits small fragments from the soluble plasma protein fibrinogen. The event results in the formation of fibrin and also activates a cross-linking enzyme. As a result of the action of the cross-linking enzyme, insoluble strands of fibrin are laid down to form a clot. Another process known as fibrinolysis is then initiated. During fibrinolysis, the enzyme plasmin is formed, and it begins to dissolve the fibrin clot. For several days, continuous formation of fibrin is balanced against its dissolution. In this way, a seal is maintained, which is limited to the site of injury, until it is replaced by healing tissue.

These normal hemostatic reactions are essential for survival in species such as man, whose blood is pumped to the tissues under high pressure. These hemostatic reactions also have a major function in other of the body's defense mechanisms. In the inflammatory response, for instance, fibrin deposited in inflamed tissues limits the spread of bacteria. In the immune response,

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*This introduction would be incomplete without acknowledging with gratitude the opportunity of the authors to review a draft report of the Evaluation of Research Needs in Hematology (1981), sponsored by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and prepared under the overall chairmanship of Ernst R. Jaffe. Of particular help was chapter VIII of the NIADDK Report, "Blood Coagulation, Platelets, and Related Phenomena," prepared under the chairmanship of Oscar D. Ratnoff, with contributions from Robert W. Coleman, Earl W. Davie, Aaron J. Marcus, Kenneth C. Robbins, Robert D. Rosenberg, and Theodore H. Spaet.

transplanted foreign tissues are rejected. Hemostasis, in fact, is a process that makes advanced forms of life possible.

Knowledge of normal hemostasis provides the foundation upon which medical science builds a true understanding of the causes and treatment of disorders associated with or resulting from abnormal bleeding and from thrombosis. These include a small but very important group of hereditary bleeding disorders, of which the hemophilias are the best known, and a larger group of acquired diseases, in which bleeding due to hemostatic failure contributes substantially to morbidity and mortality. The latter include chronic liver diseases, chronic kidney diseases, the leukemias, other malignant diseases, and diseases of autoimmunity.

Thrombosis, which is the formation of a blood clot within the lumen of a blood vessel, results from normal hemostatic reactions occurring under abnormal circumstances. Reactions that are beneficial in arresting loss of blood from a severed vessel become very harmful when they cause a blood clot to occlude the lumen of a blood vessel, with resultant cessation of the flow of blood to or from an organ or tissue. Thrombosis, which can affect arteries, veins, or the small vessels of the microcirculation, is the leading medical cause of death in the United States today. It is the immediate cause of most heart attacks and strokes.

State of Knowledge in 1972

Several reasons why circulating blood normally remains fluid were generally recognized in 1972: the lack of contact of blood with agents, such as tissue factor, capable of initiating the reactions of blood coagulation; the rapid removal of activated coagulation factors by cellular clearance in the liver; and the presence in plasma of protease inhibitors that can neutralize the activity of activated clotting factors. It was also generally appreciated that the physiology of vascular endothelial cells was different from that of other cells: the vascular endothelium maintained remarkable nonthrombogenic properties in the face of procoagulant substances and activated platelets. The reasons for the nonthrombogenic properties were unknown.

Platelets

It was known that platelets are produced primarily by megakaryocytes in bone marrow, that the time of maturation is about 4 to 5 days, and that no substantial bone marrow reserve of platelets exists. The existence of a material stimulating platelet production, thrombopoietin, was postulated on the basis of animal experiments and observations of a patient with a hereditary

disorder. The patient had repeated bouts of thrombocytopenia that were temporarily corrected by infusion of normal plasma.

The intravascular life span of platelets had been measured at 8 to 11 days, but investigators disagreed as to whether clearance of isotopically labeled platelets is exponential, which would indicate random destruction, or is linear, which would indicate age-dependent removal. Splenic pooling of one-third of the circulating platelet mass was recognized.

Many of the ultrastructural features of platelets had been described: the open canalicular system, the dense tubular system, dense bodies, granules, mitochrondria, microtubules, and microfilaments.

Ultrastructural changes of platelets after their exposure to stimuli such as collagen, thrombin, or adenosine phosphate (ADP) had been described: contraction of platelets, centralization and fusion of granules, and secretion of granule contents. A contractile protein, thrombasthin, had been isolated from platelets, and its identity with actomyosin was just becoming apparent.

It was recognized that platelets do not adhere to normal endothelium but that they rapidly adhere to components in subendothelial tissues that are exposed by a disruption of endothelial cells. The mechanism of this adhesion was not understood, and the importance of the von Willebrand factor for the reaction was not recognized.

The adhesion of platelets to each other--that is, platelet aggregation--had been studied for about 10 years, and a variety of substances, including collagen, thrombin, ADP, epinephrine, and immune complexes, had been shown to induce aggregation. ADP was thought to have an important function, but the mechanism of its action was not understood. Adenyl cyclase and phosphodiesterase, which are the enzymes that catalyze the formation and destruction of adenosine 3':5'-cyclic phosphate (cyclic AMP), had been demonstrated in platelets, and many drugs inhibiting platelet aggregation had been shown to increase the platelet content of cyclic AMP. Therefore, some, but not all, investigators believed that cyclic AMP played a regulatory function in platelet aggregation.

A prostaglandin, PGE_1, in very low concentration, was known to inhibit ADP-induced platelet aggregation. Its mechanism of action was not generally understood, although it had recently been shown that PGE_1 stimulated the activity of adenyl cyclase. The stimulated activity increased the concentration of cyclic AMP in the platelet. Nothing was yet known about prostaglandin synthesis in platelets and in endothelial cells.

The physical mechanisms by which platelets stick to each other were a mystery. Hypotheses for platelet cohesion included ADP-induced binding of platelets, by disulfide bonds, to a plasma cofactor, and the formation of bridges of actomyosin between platelets.

Blood Coagulation

It was known in 1972 that the liver synthesizes fibrinogen, four vitamin K-dependent clotting factors (prothrombin, factor VII, factor IX, and factor X), and factor V. Inferential evidence suggested that the liver also synthesizes factors XI, XII, and XIII. Why the coagulant activity of the vitamin K-dependent clotting proteins disappears from the plasma in vitamin-K deficiency was unknown. Abnormal molecules, related immunologically to normal prothrombin and to factor X, had recently been reported in the plasma of patients given oral anticoagulants, but the properties of the molecules were not further known. Fibrinogen was recognized as an acute phase protein. Very little else was known about the mechanisms controlling the synthesis of fibrinogen other than that the synthesis was independent of the concentration of fibrinogen in plasma.

From infusion experiments and from serial measurements of vitamin K-dependent clotting-factor activities after administration of oral anticoagulants, data became available for the intravascular half-times of the clotting factors. It was recognized that many of the clotting factors had remarkably short intravascular half-times--that is, 6 hours for factor VII and 10 hours for factor VIII, whereas fibrinogen had an intravascular half-time of about 4 days and factor XIII of about 12 days.

Very little was known at the molecular level about the structure of the clotting proteins other than fibrinogen and, to a lesser extent, prothrombin. The vitamin K-dependent clotting factors were known to share physicochemical properties, such as adsorption on to barium sulfate powder, but detailed knowledge of the similarities and differences of the factors was lacking. Except for its association in plasma with the von Willebrand protein, nothing was known about the molecular properties of coagulant factor VIII. Despite much effort in many laboratories, most of the clotting proteins had yet to be fully purified. The participation of prekallikrein and high molecular weight kininogen in activation of plasma by contact had yet to be discovered. The existence of two additional vitamin K-dependent plasma proteins, protein C and protein S, was unknown.

Placental, lung, and brain tissues were known to be rich sources of a factor, possibly a lipoprotein or thromboplastin, that initiates coagulation by reacting with factor VII in the

presence of calcium ions. White blood cells had been shown to acquire tissue-factor activity after exposure to endotoxin, but the acquisition was attributed to an activity of granulocytes rather than, as is now known, of monocytes. Tissue factor was known to be separable into phospholipid and apoprotein components, each devoid of coagulant activity until recombined. Tissue factor was thought to have been purified, but it is now known that the early preparations contained gross impurities.

Blood coagulation was viewed as occurring by two pathways: the intrinsic clotting reactions, initiated by contact of plasma with a negatively charged surface such as glass, and the extrinsic clotting reactions, initiated by contact of plasma with tissue factors. These two pathways were thought to merge first at the activation of factor X. Much had been learned about the sequence of the reactions in which inert precursor proteins are converted to enzymes, but knowledge of the molecular changes associated with each activation was minimal.

Contact with a negatively charged surface was thought to trigger the intrinsic clotting reactions by directly activating factor XII. Factor XII_a was then thought to activate factor XI. Participation of two additional factors in activation by contact was recognized as a possibility by some but not all investigators.

Factor XI_a had been shown to activate factor IX in a reaction requiring calcium ions but no other cofactors. A phospholipid-protein complex consisting of factor IX_a, phospholipid, calcium, and thrombin-activated factor VIII had been identified as the intrinsic activator of factor X. Thrombin activation of factor VIII had been claimed to be a prerequisite for the effective participation of factor VIII in the activation of factor X, but this view was not generally accepted. A second phospholipid-protein complex consisting of factor X_a, phospholipid, calcium, and thrombin-activated factor V was known to function as the activator of prothrombin. The importance of thrombin activation of factor V for its effect in activation of prothrombin was also recognized by a few but not most investigators.

Contact of blood with tissue factor was thought to initiate extrinsic clotting by the formation of a complex of tissue factor, factor VII, and calcium, a product that then activated factor X. The possibility of interactions between the intrinsic and extrinsic clotting systems at earlier steps in the reactions of blood coagulation was not generally appreciated.

Thrombin was known to arise from the proteolysis of prothrombin and had been partially characterized; the other fragments of prothrombin activation remained to be characterized. The view that factors VII, IX, and X represented derivatives of prothrombin rather than discrete entities had been abandoned by 1972.

The understanding of the last steps of blood coagulation--the formation, polymerization, and stabilization of fibrin--was more advanced than the understanding of the earlier steps. The basic structure of fibrinogen had been established as a dimeric molecule in which each of two identical half-molecules is made up of three peptide chains (alpha, beta, and gamma) joined at their N-terminal ends by disulfide bonds. The molecule had been visualized by electron microscopy as consisting of three modules connected in a row by a thin thread. Thrombin was known to split two small peptides from the molecule, one from the N-terminal end of the alpha chain and one from the N-terminal end of the beta chain. The resultant molecule, fibrin monomer, was then known to polymerize end-to-end and side-to-side to form insoluble fibrin.

Thrombin was also known to activate factor XIII, a two-chain plasma transamidase that catalyzes the formation of epsilon(gamma glutamyl)lysyl bonds between adjacent molecules of fibrin monomer. Cross-linking had been identified as occurring between gamma-gamma chains and between alpha-alpha chains.

Inhibitors in plasma were recognized as having a function in the regulation of blood coagulation. Both antithrombin III and alpha-2-macroglobulin had been identified as inactivators of thrombin, and antithrombin III was reported to function as the principal inactivator in serum. Alpha-2-macroglobulin had been found to inhibit the proteolytic activity but not the esterolytic activity of thrombin. Antithrombin III had also been shown to neutralize factor X_a. It had been proposed, but not yet generally accepted, that antithrombin III is the heparin cofactor of serum. The question, however, was not settled as to whether antithrombin III neutralizes thrombin by an enzymatic action or by formation of a complex. C1 inhibitor had been identified as an inhibitor of factor XII_a.

Fibrinolysis

Opinion differed as to the site of synthesis of plasminogen, with some evidence supporting synthesis in eosinophils and other evidence favoring the liver. The purification of plasminogen was technically difficult and yielded what is now known to be a degraded form of plasminogen with a residue of amino-terminal lysine. Activation of plasminogen to plasmin by urokinase had been shown to involve conversion of the single-chain precursor molecule of plasminogen to two-chain plasmin as a result of cleavage of a single arginine-valine bond in the plasminogen.

Activators of plasminogen were known to be present in most tissues and to be concentrated in two sites: lysosomal granules and vascular endothelium. Activators in endothelial cells were recognized to exist in a soluble, readily releasable form. The

release was believed responsible for the increased fibrinolytic activity in the systemic blood in response to vasoactive stimuli, such as exercise or the injection of nicotinic acid. Vascular activators were known to be cleared from the blood within minutes, presumably by liver cells, since clearance was impaired in patients with cirrhosis of the liver.

The activation by contact of factor XII in plasma had been shown to be associated with an increase in fibrinolytic activity and had provided evidence of a plasma precursor of a plasminogen activator. Urokinase, which is the plasminogen activator in urine, had been purified, and evidence for its synthesis by the kidney had been obtained. The two-step mechanism of activation of plasminogen by the bacterial substance streptokinase had been worked out with the demonstration that the initial step involves formation of a streptokinase-plasmin complex that then functions as a potent activator of human plasminogen.

A major conceptual advance, based upon knowledge of the binding of plasminogen to fibrin deposited in vessels and of the release of plasminogen activator from underlying endothelial cells, had occurred some years earlier with the description of a mechanism for control and localization of fibrinolysis. It was postulated that excess plasmin released from the local area of lysis was inactivated by circulating plasmin inactivators, which were thought to be alpha-1-antitrypsin and alpha-2-macroglobulin. Excess plasminogen vascular activator would be removed from the circulation by clearance in the liver.

The proteolytic conversion of fibrinogen to fibrin by plasmin had been described. Two large products of degradation (X and Y) and two smaller forms (D and E) had been identified. Products of fibrinogen and fibrin had been shown to interfere with hemostasis in several ways, such as interference with the action of thrombin, inhibition of polymerization of fibrin, reaction with normal fibrin monomers to form fibrin polymers with abnormally weak tensile strength, and interference with platelet aggregation.

Program Goals Through 1982

The broad Institute goals in the 1972 National Program and in the Fifth Report of the Director (1978) were:

- Determine the biochemical nature and function of the elements involved in the coagulation process and investigate the possibility of manipulating them.

- Understand the interplay of the blood coagulation system, the platelets, the vessel lining, and the properties of flowing blood.

- Increase the general understanding of the role of platelets in the mechanisms of bleeding and clotting.

More specific long-term objectives of the DBDR that evolved over the past decade, partly from recommendations of the Blood Diseases and Resources Advisory Committee, were:

- Complete the elucidation of both the intrinsic and extrinsic coagulation mechanisms, including purification and biochemical characterization of individual clotting factors and inhibitors, and determine the nature of the balance which maintains hemostasis.

- Develop an understanding of the interrelationships among the coagulation, lysis, kinin, and complement systems.

- Encourage studies of blood vessel wall biology, and develop ways to translate tissue culture results into clinical models.

- Characterize the relationships between structural components of platelets and their functions, with particular emphasis on the special properties of membranes.

- Develop further knowledge of the mechanisms involved in platelet adhesion and aggregation, in platelet release or secretion, and in the regulation of platelet cyclic nucleotide and prostaglandin metabolism.

- Encourage studies of the regulation of differentiation and maturation of megakaryocytes and of the production of platelets.

Accomplishments Through 1982

The advances in the understanding of normal hemostasis over the past 10 years have been spectacular. They derive, in very large part, from the work of biomedical investigators who have received research training support, research grant support, or both from the NIH.

Endothelium

Culturing Endothelial Cells. Development of techniques for culturing endothelial cells have permitted study of a key area--the mechanisms of the effect of vascular endothelium on the hemostatic process.

Synthesis of Prostacyclin. The discovery that endothelial cells can synthesize prostacyclin (PGI_2), which is a potent, short-lived inhibitor of platelet aggregation and is a vasodilator, represents a major advance in the understanding of the physiological mechanisms that modulate platelet reactivity in vivo. Generation of prostacyclin, from precursors intrinsic to the endothelium or provided to the endothelium by platelets, may well represent a key mechanism for maintaining the nonthrombogenic properties of the vascular endothelium.

Thrombomodulin. Endothelial cells have been shown to possess high-affinity binding sites for thrombin. Such binding may represent a physiologically important mechanism for removing thrombin from the blood, and thrombin bound to endothelium may be more rapidly inactivated by antithrombin III than is thrombin in solution. A key advance has been the isolation from endothelium of a material, given the name "thrombomodulin," that alters substrate specificity of thrombin. Thrombin bound to thrombomodulin activates protein C, an inhibitor of blood coagulation, and no longer clots fibrinogen or activates factor V.

Hemostasis by Other Endothelial Cells. Proteoglycans and heparans have been identified as components of the surface of endothelial cells. One material, heparan sulfate, is chemically related to heparin, and at high concentrations it shows anticoagulant activity. Of particular interest has been the demonstration that platelets contain an enzyme capable of liberating and degrading the heparan sulfate that is associated with the surface of endothelial cells. An enzyme with ADPase activity also found to be associated with the endothelial surface may have a function in preventing platelets from aggregating in the vicinity of intact endothelium. The plasma protease inhibitor, alpha-2-macroglobulin, has been shown to line the vascular surface of the endothelium, and it may protect the surface by binding such enzymes as thrombin, plasmin, and trypsin.

Synthesis of von Willebrand Protein. The endothelial cell has been identified as a site of synthesis of von Willebrand protein. Von Willebrand protein is released not only into the blood but also into the subendothelial tissue. The latter observation may be related to the known association between von Willebrand's disease and clinical angiodysplastic syndromes. Megakaryocytes have also been identified as a site of synthesis of von Willebrand protein.

Platelets

Culturing Megakaryocytes. The ability to culture megakaryocytes represents a landmark advance that makes in vitro

investigation possible of factors regulating differentiation and maturation of megakaryocyte, and production of platelets.

Separation of Platelets and Platelet Components. Platelet research was greatly handicapped for years because the methods used to separate platelets from plasma modified the platelets. Preparations contained partially activated platelets, not intact, unstimulated platelets. Newer techniques of filtration and density-gradient centrifugation have overcome this major problem. Techniques have also been developed to isolate, label, and separate platelet membrane glycoproteins. The development of monoclonal antibodies specific for individual membrane glycoproteins is another major technical advance in the purification of platelet components.

Platelet Adhesion to Subendothelial Tissue. Two important advances have been the identification of platelet glycoprotein I as a binding site for the von Willebrand protein, and the recognition that adhesion of platelets to subendothelium requires the participation of von Willebrand protein. An important new method for studying platelet adhesion is the development of in vitro techniques for studying the in vivo interaction between platelets and the blood vessel wall.

Calcium Flux in Platelets. A significant advance in understanding platelet function has been the identification of the release of calcium into the cytosol as a common pathway for initiating and amplifying the biochemical and structural changes in the platelets responsible for platelet aggregation.

Materials From Platelet-Membrane Lipids. Stimulation of platelets has been shown to release from platelet membrane lipids materials that are of major significance for platelet function, particularly platelet aggregating factor (PAF), phosphatidic acid, and arachidonic acid. The lipoxygenase and cyclooxygenase pathways of arachidonic acid in the platelet have been delineated, and the latter has been shown to result in the synthesis of a powerful, transient platelet-aggregating agent and vasoconstrictor, thromboxane A_2. These discoveries are major advances in the understanding of the metabolic events associated with platelet aggregation, and they have been exploited in designing approaches to antithrombotic therapy.

Platelet Cohesion in Platelet Aggregation. A breakthrough in concepts of the mechanisms of platelet cohesion has resulted from the observations that:

- Stimulation of the platelet makes available a fibrinogen receptor on its surface.

- This receptor appears to be a complex of glycoproteins II_b and III_a.
- During aggregation, fibrinogen binds to this receptor, possibly in association with a protein secreted from the alpha granules of the platelet called "thrombospondin."

Platelet Contraction. The platelet contractile apparatus has been shown to have an important function in platelet secretion, platelet cohesion, and in the consolidation of platelet aggregates. Platelet contraction has been found to be similar to the contraction of smooth muscle. Increased formation of actin filaments and binding of actin and associated proteins to the cytoskeleton have been identified as important structural changes associated with platelet activation.

Platelet Mitogenic Factor. The identification of a mitogenic factor in the alpha granules of platelets is an important discovery. It links platelet activation to the pathogenesis of atherosclerosis. Strong indirect evidence has been obtained that platelets release mitogenic factor at the site of their interaction with an injured blood vessel wall and that this release stimulates proliferation of smooth muscle cells in the vessel wall.

Thrombin Generation at Platelet Surface. Factor V_a has been shown to be released from activated platelets, to bind to the platelet surface, and, in turn, to serve as a binding site for factor X_a. Activation of prothrombin by a factor X_a-factor V_a-calcium-phospholipid complex on the surface of aggregated and activated platelets at a site of injury of the vessel wall localizes thrombin generation to the site of the injury.

Blood Coagulation

DNA for Clotting Proteins. Recombinant DNA techniques have been applied to an examination of the genes for clotting factors in what may represent a major new direction for research on hemostasis. Complimentary DNA for the three chains of fibrinogen and for prothrombin has been isolated and sequenced. Work is at an advanced state on the characterization of the genomic DNA for fibrinogen and prothrombin.

Liver-Cell Cultures. The development of techniques to maintain liver cells in culture provides important new methods for investigating clotting factor synthesis in vitro. These methods have been applied to studies of fibrinogen synthesis, and evidence suggests that a material from activated monocytes stimulates increased fibrinogen synthesis in the acute phase reaction.

Monoclonal Antibodies to Clotting Factors. Monoclonal antibodies to factor V, factor VIII, factor IX, and factor X have been prepared, and they have been used as probes of structure-function relations of these clotting proteins. They have greatly facilitated the development of methods for purification of native human factor V and factor VIII.

Proteins of Plasma Coagulation. All of the human plasma coagulation proteins, except coagulant factor VIII, have now been fully purified. This substantial accomplishment now makes research possible that is directed to expanding the knowledge of the structure and function of each of the coagulation factors.

Vitamin K-Dependent Clotting Proteins. In a landmark discovery, vitamin K was shown to be essential for gamma-carboxylation of glutamic acid residues on the NH_2 terminal segment of the vitamin K-dependent proteins of coagulation. These proteins have been characterized as zymogens that are sequentially activated, by limited proteolysis, to serine proteases during the reactions of blood coagulation. The active serine site has been identified on the carboxy-terminal segment of the proteins. Gamma-carboxyglutamic acid has been shown to be essential for the calcium-mediated binding of the vitamin K-dependent proteins to phospholipid. Two new vitamin K-dependent proteins have been discovered, protein C and protein S, and a function for protein C in the regulation of blood coagulation has been identified.

Structure of Coagulation Proteins. The rapid progress in delineating the primary structure of the coagulation proteins is another example of the remarkable advances in biochemistry of the past 10 years. The complete amino acid sequence is now known for human fibrinogen and prothrombin. Partial amino acid sequences are known for human factor X, factor IX, and protein C.

Molecular Changes During Activation. The sites of limited proteolysis and resultant molecular changes have been elucidated for the activation of a large majority of the coagulation proteins: factor XII, prekallikrein, high molecular weight kininogen, factor XI, factor IX, factor VII, factor X, prothrombin, fibrinogen, and factor XIII.

Contact Initiation of Intrinsic Clotting. Prekallikrein and high molecular weight kininogen have been identified as proteins participating in the activation of blood coagulation by contact with negatively charged surfaces. The reciprocal activation of factor XII and prekallikrein has been demonstrated, and a role for high molecular weight kininogen as a carrier for both prekallikrein and factor XI has been identified. Mechanisms in which activation of plasma results in kinin activity and in the activation of plasminogen have also been delineated.

Tissue Factor Activation of Coagulation. A significant advance in understanding the mechanisms that initiate blood coagulation has resulted from the demonstration that the complex of tissue factor and factor VII activates factor IX as well as factor X. Increasing evidence suggests that the activation of factor IX by tissue factor and factor VII represents a major physiological pathway for initiating blood coagulation. Evidence has also been obtained for a feedback reaction in which a complex of tissue factor and native factor VII initiates activation of factor IX and factor X and in which these activated enzymes, in turn, convert native factor VII to factor VII_a, with a resultant manyfold increase in coagulant activity.

Activation of Prothrombin. The binding sites on the prothrombin molecule for phospholipid and for factor V_a have been identified, and the effect of calcium ions upon the conformation of the molecule is now better understood. The sites of proteolysis in prothrombin induced by factor X_a and by thrombin have been elucidated.

Functions of Factor XIII. In addition to catalyzing the cross-linking of fibrin, factor $XIII_a$ has been shown to catalyze the cross-linking of fibronectin to fibrin, of fibronectin to collagen, and of alpha-2-antiplasmin to fibrin. These observations provide important information for understanding the mechanisms of normal healing of wounds and the acquisition of resistance of fibrin to fibrinolysis over time.

Function of Protein C. Activated protein C (protein C_a) has been shown to inactivate factor V_a and factor $VIII_a$. The function of a putative plasma inhibitor of protein C_a has been reported to be reduced in the rare hereditary disorder of combined factor V and factor VIII deficiency. A family was identified in which familial thrombotic disease was associated with reduced plasma protein C antigenic levels. These observations strongly suggest that protein C has a hitherto unrecognized major function in hemostasis.

Antithrombin III. Simplified affinity-chromatographic techniques have been developed that permit purification of antithrombin III in good yield. The complete primary structure of antithrombin III has been determined as well as many of its physiochemical features. The binding sites on the molecule for heparin have been identified. Important progress has been made in the detailed molecular analysis of the interaction of antithrombin III and serine protease coagulation enzymes in the presence and in the absence of heparin.

Alpha-2-macroglobulin. The protease inhibitor alpha-2-macroglobulin has been found to be made up of four similar chains. Its primary sequence has been partially determined, and its

binding of proteases has been shown to be associated with subsequent cleavage of the polypeptide chain of the inhibitor. Of particular interest has been the observation that a small fraction of bound proteases retain their activity and are protected from neutralization by other protease inhibitors.

Fibrinolysis

Plasminogen. In a series of major advances, plasminogen has been purified, its complete amino acid sequence has been determined, the lysine binding sites on the molecule have been identified, and the differences in the activation properties of native plasminogen (Glu-plasminogen) and degraded plasminogen (Lys-plasminogen) have been partially delineated. A key function for the lysine binding sites in binding of plasminogen to fibrin and of alpha-2-antiplasmin to plasmin and plasminogen has been established.

Vascular Activator. A major step in understanding the mechanisms that regulate the initiation of fibrinolysis has resulted from the observation that the affinity of vascular activator for plasminogen increases remarkably in the presence of fibrin. This increased affinity has not been observed in other plasminogen activators, such as urokinase or streptokinase. Vascular activator should, therefore, be an ideal agent for inducing therapeutic fibrinolysis, and efforts to develop vascular activator for therapeutic use are under way.

Alpha-2-antiplasmin. A breakthrough in the knowledge of the control of fibrinolysis has resulted from the identification of alpha-2-antiplasmin as the major plasma inhibitor of plasmin. Alpha-2-antiplasmin has been shown to bind to the active center of plasmin and to its lysine binding sites; alpha-2-antiplasmin also binds to the lysine binding sites of plasminogen. Factor XIII has been shown to cross-link alpha-2-antiplasmin to fibrin. Patients have been discovered with severe, hemophilia-like bleeding due to a hereditary deficiency of alpha-2-antiplasmin.

Histidine-Rich Glycoprotein. A histidine-rich protein in plasma has been identified that binds to the lysine binding sites of plasminogen, competing with alpha-2-antiplasmin inhibitor for such binding sites. Thus, a second mechanism may exist for regulating the amount of plasma plasminogen available for participation in fibrinolysis.

State of Knowledge in 1982

The remarkable accomplishments listed above changed the understanding of hemostasis in 1972 from a largely descriptive one

to one in which individual reactions are being defined in precise, molecular terms. This advance has resulted partly from the development of important new techniques, including application of recombinant DNA and of monoclonal antibodies; culture methods for endothelial cells, liver cells, and megakaryocytes; and affinity-chromatographic and gel-sieving techniques for isolating and separating proteins. Platelets can now be prepared from plasma in their unstimulated state, and the individual plasma clotting factors, except coagulant factor VIII, can be purified from plasma without activation. Chromatographic techniques and assays involving the release of activated tritiated peptides have been developed that make it possible to measure individual steps of activation in complex reaction mixtures and to calculate kinetic constants in both purified and whole-plasma systems.

Endothelium

It is now appreciated that the nonthrombogenic properties of vascular endothelium result from dynamic processes that involve the synthesis of prostacyclin, the enzymatic activity of an endothelial-surface ADPase, the binding of thrombin to one or more binding sites with resultant modulation of its substrate specificity, and the possible interaction between antithrombin III and heparans on the endothelial cell surface. It is also now recognized that a second protease inhibitor, alpha-2-macroglobulin, lines the inner surface of blood vessels. It provides another potential mechanism for protecting endothelium from the actions of proteases. Endothelial cells have also been established as the site of synthesis of two key proteins for hemostasis: von Willebrand protein and plasminogen activator. Studies should now be undertaken to delineate the interaction and relative importance of each of these endothelial-related processes of hemostasis.

Platelets

Although it is now possible to study thrombopoiesis by the use of methods of megakaryocyte culture, such studies are still at an early stage. A colony-stimulating factor for megakaryocytes has been identified in the urine of patients with idiopathic thrombocytopenic purpura, and the factor has been shown to be distinct from erythropoietin. In addition, a technique for labeling platelets with ^{111}Indium has simplified the methodology for measuring platelet turnover. The pattern of disappearance of isotopically labeled platelets in normal individuals has been shown to follow a linear model more closely than a curvilinear one. This finding indicates that removal of platelets is related primarily to age.

Platelet adhesion to subendothelial tissue is now known to involve binding sites on collagen and other subendothelial materials, the large multimers of von Willebrand protein, and glycoprotein I of the platelet surface membrane. In experimental models, the relative importance of the von Willebrand multimers for platelet adherence has been shown to vary with the rate of blood flow and resultant wall shear stress. Evidence is accumulating that the platelet must be stimulated by exposure to collagen and thrombin before the von Willebrand protein can bind to glycoprotein I. Little is yet known at the molecular level, however, about the binding sites on subendothelial tissues, the binding sites on the platelet surface membrane, and the reactions with the multimers of the von Willebrand protein.

Regulation of calcium flux within the platelet cytosol is now thought to be involved in platelet aggregation. How, at the molecular level, such initiating and amplifying stimuli as collagen, thrombin, ADP, and thromboxane A_2 interact with platelet surface membrane materials and initiate the reactions that alter the level of cyclic AMP is still largely unknown.

It is now known that calcium released into the platelet cytosol binds to a protein, calmodulin, and that the calcium-calmodulin complex activates the enzymes that are involved in platelet aggregation, including platelet phospholipases. Lipid-derived materials with platelet-aggregating properties are formed: thromboxane A_2, phosphatidic acid, and PAF. The reactions, through the cyclooxygenase pathway, in the transformation of arachidonic acid, which is released from platelet membrane phospholipids, to thromboxane A_2, are now understood in detail. A second pathway of arachidonic acid oxidation, by lipoxygenase, has also been identified within platelets, but a hemostatic function for its products has not been identified. The relative importance of thromboxane A_2, phosphatidic acid, and PAF for platelet aggregation requires further evaluation. In fact, very little is known about PAF. Moreover, whether thromboxane A_2 directly functions primarily as a platelet aggregator or acts indirectly, through release of dense granule ADP, remains to be settled.

It is now well established that stimulated platelets secrete materials from their alpha granules and dense bodies during aggregation. Some materials, such as ADP, thrombospondin, beta-thromboglobulin, and PF4, are unique to the platelet, whereas others, such as factor V, von Willebrand protein, fibrinogen, and fibronectin, are present in far greater amounts in the plasma. Important hemostatic functions for secreted ADP and thrombospondin have been identified. Hemostatic functions for secreted PF4 and for beta-thromboglobulin are yet to be identified, although PF4 has been shown to modify the enzymatic activity of collagenase and elastase. Secreted platelet factor V, fibrinogen, fibronectin, and von Willebrand protein have each been shown to bind to the

platelet surface membrane. It is not yet clear whether selective binding of these platelet-secreted materials occurs during hemostasis--that is, whether these platelet coagulant proteins have a unique function that is unlike their plasma counterparts.

The mechanism of platelet contractions has been shown to resemble the contractions of smooth muscle. Activation, by calcium-calmodulin, of myosin light-chain kinase initiates the contraction of platelet actin, and phosphorylation of myosin light-chain kinase initiates the relaxation of platelet actin. Actin filaments increase when platelets are activated and are bound, with related proteins, to the cytoskeleton of the platelet. The contractile mechanism of platelets is recognized as participating in the formation of platelet pseudopods, in the centralization of platelet granules that precedes secretion of granule contents, and in the orderly wave of contraction that consolidates the platelet aggregate.

The mechanism by which individual platelets stick to each other during platelet aggregation is beginning to be understood at the molecular level. Activation of platelets is now known to alter the spatial relationship of platelet surface glycoproteins II_b and III_a. The alteration results in the formation of a complex that serves as a binding site of fibrinogen. Fibrinogen binds to the platelet surface as does the high molecular weight protein thrombospondin, which is secreted from platelet alpha granules after stimulation of the platelet. How binding of fibrinogen and binding of thrombospondin, acting together or separately, then cause platelets to adhere to each other is not yet clear.

Blood Coagulation

The liver is now recognized as the site of synthesis of all of the blood coagulation factors except coagulant factor VIII, whose site of synthesis remains unknown. Complementary DNA for human fibrinogen and prothrombin has been isolated and sequenced, and the techniques that were used are applicable to the other blood coagulation proteins. The increased synthesis of fibrinogen that occurs during the acute phase reaction is believed to be mediated, at least partially, by material released from activated monocyte-macrophages. Almost nothing is yet known, however, about the mechanisms of normal synthesis of the blood coagulation factors, and little has been learned about the mechanisms of catabolism of the blood coagulation proteins.

All the blood coagulation factors, except coagulant factor VIII, have been fully purified in their native state, and the general features of their structures have been delineated. All the vitamin K-dependent clotting proteins have been shown to

possess a newly discovered amino acid, gamma-carboxyglutamic acid, which is now known to bind calcium, with a resultant structural change in the protein and an associated alteration in phospholipid binding capacity. The complete amino acid sequences of human fibrinogen and of human prothrombin have been established, and partial amino acid sequences are known for factor X, factor IX, and protein C. Tissue factor apoprotein from bovine brain has been purified.

The tissue factor activity of white blood cells has been found to be associated with activated monocytes and, in most circumstances, to require for its development the participation of T lymphocytes.

Generation of thrombin during blood coagulation is now recognized as taking place in a sequence of reactions in which an initial inert precursor is converted into an active serine protease enzyme, which, in turn, activates the next inert precursor. Amplification occurs, so that formation of a few molecules of an initial serine protease enzyme eventually leads to the formation of many molecules of thrombin. Activation of each serine protease enzyme results from limited proteolysis of the native molecules. The molecular changes resulting from the limited proteolysis of each molecule have been characterized. Cofactors, which are tissue factor, high molecular weight kininogen, factor VIII, and factor V, participate at different steps in the sequence of reactions. One function that the cofactors serve is to help localize reactions at surfaces. Two of the cofactors, factor V and factor VIII, require activation by traces of thrombin for their function. The molecular changes resulting from their activation are not yet well understood.

In vitro contact of blood with a negatively charged surface initiates blood coagulation through reactions that involve four plasma proteins: factor XII, prekallikrein, factor XI, and high molecular weight kininogen. Factor XI and prekallikrein circulate in plasma as complexes with high molecular weight kininogen. Factor XII is attracted to the negatively charged surface, as is high molecular weight kininogen. Factor XI and prekallikrein follow. How the first active molecules of proteinase are formed is not known; one view holds that native factor XII may possess trace enzymatic activity able to initiate subsequent reactions. Thus, activated factor XII activates prekallikrein to kallikrein, and kallikrein, in turn, activates factor XII, with formation of both a large activated form, which is alpha-factor XII_a, and a small activated form, which is beta-factor XII_a. The former remains on the surface and readily activates factor XI. The latter, which is a poor activator of factor XI, leaves the surface and readily activates prekallikrein. The vascular subendothelium contains material capable of initiating the activation reactions resulting from contact, but these reactions may not be important

for physiological hemostasis since patients with hereditary deficiencies of factor XII, prekallikrein, and high molecular weight kininogen do not bleed abnormally.

Through generation of kallikrein, the contact reactions also result in the formation of kinins from both high and low molecular weight plasma kininogens. Moreover, factor XII_a, kallikrein, and factor XI_a have all been found to activate plasminogen. The physiological significance of this interrelationship of activation of blood coagulation, generation of kinins, and activation of fibrinolysis remains speculative. As yet, a relation between factor XII deficiency and a clinical manifestation of disease remains to be established definitively.

When blood contacts tissue factor, a complex composed of a tissue factor and native factor VII is formed, which appears to possess just enough coagulant activity to initiate the clotting process. This complex is now known to activate not only factor X but also factor IX. Both activations appear to be required for normal hemostatic function. Indeed, the contrast between the severe bleeding of factor IX deficiency and the mild bleeding of factor XI deficiency suggests that the activation of factor IX by the complex of tissue factor and factor VII may be more significant for normal hemostasis than is the factor XI_a activation of factor IX.

Activation of factor VII to factor VII_a as a result of limited proteolysis makes possible the formation of a complex composed of tissue factor and factor VII_a. This complex possesses coagulant activity manyfold greater than the coagulant activity of the complex of tissue factor and native factor VII. Known activators of factor VII include factor XII_a, factor IX_a, and factor X_a. Thus, the tissue-factor pathway of coagulation contains a feedback loop in which the first factor IX_a and factor X_a that are formed can enhance substantially the rate of subsequent formation of factor IX_a and factor X_a. The significance of this feedback loop for normal hemostatic function requires further evaluation.

Factor IX_a forms a calcium-dependent complex that together with phospholipid and thrombin-activated factor VIII (factor $VIII_a$) is a potent activator of factor X. Undoubtedly, the platelet surface membrane provides the phospholipid for this complex, and, therefore, the complex should form on the platelet surface membrane. Nevertheless, evidence of binding of factor $VIII_a$ and factor IX_a to the platelet surface has yet to be obtained.

In an analogous reaction, factor X_a forms a calcium-dependent complex together with phospholipid and thrombin-activated factor V (factor V_a) to activate prothrombin. Despite its capability of

binding by its gamma-carboxyglutamic acid groups to platelet-surface phospholipid, factor X_a does not bind to the platelet surface unless an additional binding site, provided on the surface by factor V_a, is also available. The prothrombin activator cleaves prothrombin on the platelet surface to form thrombin. Cleavage of prothrombin involves loss of the amino-terminal fragment of the prothrombin molecule--that is, the segment of the molecule containing not only the gamma-carboxyglutamic acid molecules, by which prothrombin binds to platelet-surface phospholipid, but also the binding site on prothrombin for factor V_a. As a result, thrombin does not remain bound to the platelet surface.

Thrombin cleaves two fibrinopeptides from fibrinogen. The cleavage exposes binding sites on the alpha and beta chains that permit the resultant fibrin molecules to polymerize to form insoluble fibrin. Thrombin also cleaves a polypeptide from the alpha chains of factor XIII. This cleavage thus activates factor XIII, which then catalyzes the cross-linking, by transamidation, of fibrin to fibrin, of alpha-2-plasmin inhibitor to fibrin, of fibronectin to fibrin, and of fibronectin to collagen. In the process of healing, these reactions stabilize fibrin, increase its resistance to fibrinolysis, and facilitate the movement of fibroblasts into the area of injury.

The understanding of the processes that regulate the reactions of blood coagulation has grown. Limited amounts of available factor VIII and available factor V appear to represent an important regulatory mechanism. Although thrombin must activate both factor VIII and factor V for their effective participation in the reactions of blood coagulation, the resultant activated forms, factor $VIII_a$ and factor V_a, are unstable molecules whose coagulant activity is rapidly lost. Moreover, when thrombin is bound to the endothelial-cell binding site thrombomodulin, thrombin in low concentration can activate protein C. Protein C_a, in the presence of calcium and phospholipid, very rapidly inactivates factor $VIII_a$ and factor V_a. Thus, there are two mechanisms that limit the availability of factor $VIII_a$ and factor V_a during hemostasis. The recent report of a family with thrombotic disease and reduced plasma levels of protein C antigen focuses attention upon the potential physiological importance of the protein C_a mechanism.

Antithrombin III also functions in the regulation of the reactions of blood coagulation by serving as the primary plasma inhibitor of thrombin and also of factor X_a and factor IX_a. Whether antithrombin III also contributes significantly to the inactivation of the coagulant function of the complex of tissue factor and factor VII_a during physiological hemostasis is not yet clear. It is suspected, but not established, that antithrombin III activity is enhanced in vivo by interaction with proteoglycans

or heparans on the surface of endothelial cells. Alpha-2-macroglobulin has been shown to inhibit thrombin, plasmin, and kallikrein, but a function for alpha-2-macroglobulin in the physiologic regulation of hemostasis has not been identified. C1 inhibitor appears to be the primary inactivator of the proteases involved in contact activation of blood coagulation. Evidence also exists for a plasma inhibitor of protein C_a, although it is yet to be isolated.

Fibrinolysis

The understanding of fibrinolytic mechanisms has advanced remarkably in the past several years. It has been established that plasminogen is synthesized primarily in the liver. Plasminogen is now known to circulate in plasma partially as complexes: with alpha-2-antiplasmin, with a second plasma protein called histidine-rich glycoprotein, and, to a limited extent, with fibrinogen. How this multiple binding affects the availability of plasminogen for fibrinolysis remains to be clarified. It is known, however, that in contrast to its weak affinity for fibrinogen, plasminogen has a strong affinity to bind with fibrin. Thus, the conversion of fibrinogen into fibrin is associated with adsorption and accumulation of plasminogen on fibrin.

Human plasminogen can be purified by simplified affinity-chromatographic techniques. The complete amino acid sequence of plasminogen has been established. It is now recognized that plasminogen possesses lysine binding sites that are critically important for the binding of plasminogen to fibrin and also for the neutralization of free plasmin by alpha-2-antiplasmin. Recent peptide mapping studies have established the importance, in plasminogen, of a single tryptophan residue for its strong lysine binding site.

Native plasminogen is a single-chain molecule with an NH_2-terminal glutamic acid (Glu-plasminogen). Limited digestion by plasmin degrades native plasminogen by splitting off an NH_2-terminal polypeptide. This cleavage results in molecules with lysine, valine, or methionine as the NH_2-terminal amino acid; the molecules are referred to collectively as Lys-plasminogen. Lys-plasminogen is more readily activated by plasminogen activators than is Glu-plasminogen. Thus, plasminogen activation contains a feedback loop in which the initially formed plasmin can accelerate subsequent plasmin generation. The significance of this feedback activation for physiological recirculation of fibrinolysis is not yet clear.

Plasminogen activators convert plasminogen to plasmin through cleavage of a single Arg-Val bond. In the conversion, the single-chain precursor molecule is split into a two-chain serine protease

whose active site is located on the carboxy-terminal, or light-chain segment, of the activated molecule. The two physiological pathways of plasminogen activation that have been defined are an intrinsic pathway, which involves only the components that are present in precursor form in the blood, and an extrinsic pathway, which involves the release of an activator from tissue, primarily from endothelial cells, into the blood. The intrinsic pathway involves three contact-activation factors of the blood-coagulation reactions: factor XII_a, kallikrein, and factor XI_a, each of which has been shown to activate plasminogen. C1 inactivator has been identified as the main inhibitor of these intrinsic plasminogen activators. Presently, however, the intrinsic plasminogen activators are thought to have only a minor function in the physiologic regulation of fibrinolysis.

Vascular plasminogen activator, which is released from endothelial cells after certain stimuli or trauma, functions as the major physiological activator of fibrinolysis. In this reaction, a vasoactive stimulus initiates the release of vascular plasminogen activator, as in the vasodilatation that follows the intravenous injection of nicotinic acid. When fibrin is deposited within a vessel, the underlying endothelium apparently releases vascular plasminogen activator. The mechanism of the release is not clear. In tissue culture, thrombin appears to inhibit such release from endothelium. Protein C_a, however, has recently been reported to stimulate endothelial cells to release this activator.

Neurohumoral regulation of release by a putative plasminogen-activator-releasing hormone similar in properties to vasopressin has been postulated. Catecholamines also initiate release of vascular plasminogen activator.

The properties of vascular plasminogen activator are rapidly being characterized. It is a single-chain protein, with molecular weight of 77,000, which is readily converted by limited proteolysis into a two-chain molecule of increased in vivo reactivity. A remarkable property of this activator has recently been recognized. In the absence of fibrin or other denatured protein, it is an inefficient activator of plasminogen, whereas in the presence of fibrin or other denatured protein, it is highly efficient. These disparate reactions provide a key mechanism for the control of fibrinolysis: the process is limited to sites where it is needed for dissolving fibrin or other denatured tissue proteins. Moreover, vascular plasminogen activator entering the general circulation has the very short intravascular half-time of only 3 minutes. This interval reflects its rapid clearance by the liver.

Alpha-2-antiplasmin, a single-chain glycoprotein with a molecular weight of 70,000, is now recognized as the physiological plasma inhibitor of plasmin. It is synthesized in the liver, is a

weak acute-phase reactant, and has an intravascular half-time of 2.5 to 3 days. It inactivates free plasmin very rapidly in a reaction that involves its binding to both the active enzymatic sites and the lysine binding sites of plasmin. Therefore, alpha-2-antiplasmin is less effective in inhibiting plasmin bound to fibrin, because plasmin bound to fibrin has both its lysine binding and enzymatic sites occupied. This difference in inhibitory efficiency may be another important mechanism for confining fibrinolysis to sites of deposited fibrin. By competition for the lysine binding sites of plasminogen, alpha-2-antiplasmin may also regulate fibrinolysis by interfering with the adsorption of plasminogen to fibrin. Moreover, factor XIII has recently been shown to cross-link alpha-2-antiplasmin to fibrin in a reaction that could be related to why fibrin deposited in vessels becomes increasingly refractory to lysis over time.

Program Goals 1982 to 1987

- Increase the understanding of blood vessel wall biology as it is related to hemostasis through: studies of the contribution of platelets to the maintenance of blood vessel wall integrity; investigations of the plasma membrane proteins of endothelium, smooth muscle cells and fibroblasts, and alterations in these proteins that lead to activation of coagulation and elaboration of platelet coagulant activity; studies of the interaction with underlying blood vessel wall components of von Willebrand protein and other materials secreted by endothelial cells; qualitative and quantitative studies of the types of collagen produced by vascular cells and their reactivity in hemostatic processes.

- Encourage studies of rheological factors of hemostasis, including the development of models for evaluating rheological effects upon platelet-vessel wall interactions, and studies of how alterations of endothelial cell surface proteins may affect rheological parameters.

- Delineate the mechanisms, possibly by megakaryocyte culture techniques, regulating differentiation of stem cells into megakaryocytes, the maturation of megakaryocytes, and platelet production.

- Delineate at a molecular level the reactions that result in platelet adhesion, including the interaction of multimers of von Willebrand protein with binding sites of subendothelial tissue and with glycoprotein I of the platelet membrane.

- Continue studies at a molecular level of the structural properties and biochemical reactions of platelets important for platelet aggregation, including: investigations of the structural and functional properties of platelet membrane glycoproteins; studies of the structure and biochemical reactions of the mechanism of platelet contraction; studies of cyclic nucleotides, prostaglandins, and other lipid-derived materials such as PAF and lipoxygenase pathway products in the regulation of platelet function; and studies of the function of materials secreted from stimulated platelets.

- Continue investigations of reactions between platelet surface membrane components and blood coagulation factors, including elucidation of the alterations in the platelet surface membrane that make binding sites for blood coagulation factors available.

- Continue the application of recombinant DNA techniques to studies of the blood coagulation and fibrinolytic proteins in order, ultimately, to characterize the genomic DNA for each of these proteins.

- Stimulate studies of the control of synthesis of the blood coagulation proteins and of mechanisms of their catabolism. Such studies should utilize whole animal, organ perfusion, and tissue culture systems. Of particular importance may be studies of synthesis that utilize liver cell cultures.

- Continue, for each of the plasma coagulation proteins, to determine the primary amino acid sequence, the three-dimensional structure, and the structural properties that determine their specific biochemical functions in coagulation.

- Increase the understanding of the processes that initiate, amplify, and regulate the known blood coagulation reactions, through detailed kinetic studies, with purified coagulation proteins, of the individual reactions of the blood coagulation cascade.

- Search further for reactions of blood coagulation, including a mechanism for activation of factor XI independent of other contact-active factors, and a possible function of protein S in hemostasis.

- Delineate at a molecular level the mechanisms by which endothelial cell surface materials may influence the blood coagulation reactions and, in turn, activated coagulation proteases may affect endothelial cell function, with

particular attention to reactions involving thrombin and protein C.

- Investigate the properties and activities of tissue factor, including: purification and structural analysis of human tissue factor apoprotein from different cellular sources; location and organization within the cell membrane of molecules with tissue factor activity; and mechanisms by which tissue factor becomes available on cell surfaces to interact with plasma coagulation factors.

- Continue structure-function studies of fibronectin, of its possible role in hemostasis, and of how it links the hemostatic reactions to other functional systems in plasma or on cell surfaces.

- Define in molecular detail the biochemical steps whereby antithrombin III and alpha-2-macroglobulin inactivate each of the plasma proteases with which they react, including the manner in which specific ligands such as heparin and amines modulate the reactivity of the inhibitors.

- Determine the mechanisms regulating the hepatic synthesis of plasminogen and alpha-2-antiplasmin and the physiological processes determining their rate of catabolism; and evaluate the mechanisms, including neurohumoral and endocrine influences and effects of activated coagulation factors that control the synthesis and release of vascular plasminogen activator.

- Continue structural studies of the proteins of fibrinolysis, including studies of the three-dimensional structure of plasminogen and a determination of the primary structure of vascular plasminogen activator and alpha-2-antiplasmin.

- Broaden the understanding of the biochemical events involved in the initiation, regulation, and control of fibrinolysis, including: the molecular basis for the enhanced affinity of plasminogen for fibrin; the molecular basis of the enhanced affinity of vascular plasminogen activator for plasminogen induced by fibrin or other denatured protein; the significance of binding of plasminogen to alpha-2-antiplasmin and histidine-rich glycoprotein for regulation of fibrinolysis; and, at the molecular level, the biochemical steps involved in the inhibition of plasmin by alpha-2-antiplasmin.

- Increase knowledge of the links between hemostasis and other key biologic phenomena, including: roles of platelet-derived materials in modulating inflammatory and

repair processes; effects of surface-mediated reactions, involving the contact factors, in inflammatory responses; interactions between the fibrin gel, fibronectin, connective tissue elements, factor XIII-catalyzed reactions, and the fibrinolytic process in wound healing; participation of the hemostatic reactions in immunologic responses; and interactions between the complement system and the coagulation and fibrinolytic reactions.

Research Activities 1982 to 1987

Current Activities

It is difficult to place many of the projects currently supported by the thrombosis and hemostasis program in a single research category. A project focused upon the cause for abnormal platelet function in a particular hereditary qualitative platelet disorder, for example, may not only provide knowledge about that disorder, but more importantly, it may also yield knowledge essential for an understanding of normal hemostasis and of the function of platelets in the pathogenesis of thrombosis. With this in mind, one can list 168 separate projects currently supported by the thrombosis and hemostasis program as concerned in a substantial way with the elucidation of the factors and processes of normal hemostasis. These projects encompass a very wide range: traditional approaches to purification and characterization of proteins of the coagulation and fibrinolytic systems; investigations of the interrelations between the hemostatic process and the complement and kinin systems; and the use of new techniques in molecular and cellular biology to study cell growth and function and protein synthesis. In many projects, monospecific antibodies generated through hybridoma technology are being used to enhance capabilities of purification and to study structure-function relationships in detail.

Platelets. The development of platelets, their function as cells, and their participation in surface-related phenomena are the subject of approximately 70 DBDR projects. Different aspects of the responses of platelets to activation are under extensive study. Over 15 projects are focused on the function of cyclic nucleotides and calcium in the control of platelet function and on the mechanisms of aggregation and release. Platelet lipid metabolism is under study in projects ranging from the relative functions of phospholipases A and C in arachidonate production, to the effects of thromboxane A_2 and endothelial cell prostacylin upon the interaction of platelets with each other and with the vessel wall. Eleven projects are directed toward understanding the platelet surface membrane and its receptors and the relation

of specific surface glycoproteins to platelet function. The contractile proteins and contractile process of platelets are being investigated in at least seven laboratories.

Although new culture techniques make it possible to study in vitro the mechanisms controlling the differentiation and maturation of megakaryocytes, such studies are still few. The potential of this technique for studies of the physiology of platelet production in normal and pathologic states has yet to be fully exploited.

Coagulation. The so-called contact factors of blood coagulation are being extensively studied in at least 10 laboratories. Overall objectives include a delineation of the biochemical events associated with activation and control of factor XII-dependent pathways for intrinsic coagulation, and a delineation of the function of factor XII-dependent pathways in fibrinolysis and the generation of kinins. Projects are directed to specific factors and their interactions and also to the systems and their relation to each other in both normal and disease states.

The tissue factor-factor VII, or extrinsic, pathway is an area of great importance, but it is receiving minimal support at present. Only four projects are directed toward characterization of tissue factor, regulation of the tissue factor pathway, and the relation between this pathway and the contact activation pathway. It appears from these studies, however, that a new concept of the physiologic coagulation process may be emerging.

Studies of the structure-function relationships of the vitamin K-dependent clotting factors are under way in at least 14 laboratories. Several groups are investigating how the binding of metal ions to the gamma-carboxyglutamic acid residues on these proteins alters their three-dimensional structure. The function of the gamma-carboxyglutamic acid groups in the binding of these proteins to phospholipid is also being studied. Continued substantial progress in the understanding of the structure-function relationships of the vitamin K-dependent proteins may be anticipated. At least three groups are studying the interaction of factor IX, factor VIII, and platelets.

Twenty-three projects are concerned with one or more aspects of the reactions in which factor X_a, factor V_a, phospholipid, and calcium ions convert prothrombin to thrombin. The factors that regulate the rate and extent of production of factor X_a and of thrombin are rapidly being clarified. With the purification of human factor V and its partial characterization, one may anticipate understanding at the molecular

level the regulatory function of this protein in prothrombin activation.

Fibrinogen continues to be studied extensively in 19 currently supported projects. With the determination of its complete primary structure, research emphasis has shifted toward understanding the three-dimensional structure of the molecule and of the domains involved in polymerization and in cross-linking by factor $XIII_a$. Also under investigation are the properties of factor XIII, the action of factor $XIII_a$ upon plasma fibronectin, and the interaction of fibrin and fibronectin.

The proteins that inhibit blood coagulation--the protease inhibitors, which are antithrombin III and alpha-2-macroglobulin, and the recently discovered vitamin K-dependent protein, which is protein C--are under study in a number of laboratories. Recent evidence suggesting that protein C may be an important regulator of both blood coagulation and fibrinolysis has excited a number of investigators, and the number of studies in this area should increase. Substantial advances in the understanding of protein C in hemostasis may be anticipated in the next 5 years.

It has become increasingly apparent that certain cells have key functions in hemostasis. Interest is growing in three areas: endothelial cell biology; the participation of platelets in the initiation of coagulation; and the relation of monocytes and other cells to procoagulant activities. Although all three areas are increasingly recognized as deserving emphasis, present activity in each is only moderate.

Studies of the synthesis and catabolism of the blood coagulation proteins have lagged far behind studies of their structure and function. The synthesis of fibrinogen, however, is receiving increasing attention, and six laboratories are currently being supported in studies of mechanisms that regulate it. Several laboratories are also studying synthesis of the vitamin K-dependent proteins, particularly the function of vitamin K itself and of the enzymes and coenzymes of the carboxylation reaction, which is vitamin K-dependent and posttranslational. Studies of mechanisms of fibrinogen catabolism are being supported in one laboratory.

A major effort is being supported in one laboratory on the characterization of the genomic DNA for fibrinogen and prothrombin. One may anticipate continued progress in this new and important area and the likely spread of interest to a number of laboratories in characterizing the genes for clotting factors.

Fibrinolysis. Twelve laboratories are currently being supported in studies related to one or more components of the fibrinolytic system: plasminogen, plasminogen activators from tissues and cultured cells, urokinase, streptokinase, and inhibitors of fibrinolysis, especially alpha-2-antiplasmin. The studies employ biochemical approaches to the physical and enzymatic characterization of the different components, and cell-culture approaches to the synthesis and release of plasminogen activator. The release of plasminogen activator from endothelium by protein C_a is also being studied in animals.

New Activities

Most new activities of the next 5 years are expected to stem from current ones. As examples, one may confidently anticipate:

- Development of increasing numbers of monoclonal antibodies specific to platelet surface glycoproteins, and use of these antibodies in defining the structure and function of these proteins and their relation to surface binding sites for such physiologically significant materials as collagen, ADP, thrombin, epinephrine, prostacyclin, and thromboxane A_2.

- Elucidation of the primary structure and detailed structure-function relationships of most if not all of the blood coagulation proteins, and of the alterations in their tertiary structure that are induced by activation.

- Characterization of the structure and mechanism of action of the putative plasma protein C_a inhibitor.

- Determination of the primary structure and the structure-function relationships of vascular plasminogen activator.

- Determination of the kinetic effects of antithrombin III activity on the interaction of antithrombin III with proteoglycans and heparan sulfate that occurs on endothelial cell surfaces.

- Application of recombinant DNA techniques to characterization of the genomic DNA of coagulant factor VIII.

The time also seems ripe for a shift of emphasis from studies concerned primarily with the blood coagulation reactions in soluble systems to studies involving reactions at cell surfaces and the interaction of cell surface moieties with the blood

coagulation proteins and their inhibitors. Three areas deserving substantially increased activity have been identified:

Blood Coagulation Reactions at the Platelet Surface. The factor X_a-factor V_a-phospholipid-calcium-prothrombin reaction on the platelet surface has already been extensively studied. One may anticipate that within the next 5 years the factor IX_a-factor $VIII_a$-calcium-phospholipid-factor X reaction on the platelet surface will be characterized. The characterization should be of particular importance in understanding the severity of bleeding in hemophilia. One may also hope that studies of activation of factor XI on the platelet surface may finally identify the putative mechanism of activation for factor XI that bypasses the factor XII-dependent pathway.

Tissue Factor. Only a few research laboratories are currently involved in studies of tissue factor, including its purification from human tissues, structural analysis, location and organization within cell membranes, mechanisms determining its availability on the surfaces of different types of cells, and kinetics of the tissue factor-factor VII initiation of coagulation on cell surfaces. Advances will require increasing interdisciplinary efforts of experts in cell membrane biology and in hemostatic reactions in blood coagulation. The DBDR has therefore planned a workshop in September 1982 that will bring these two groups of investigators together to discuss cells and hemostasis. It is hoped that the workshop will encourage a major increase in tissue-factor-related research through the use of targeted research funds. This project deserves a very high priority.

Interactions Between Cells of the Blood Vessel Wall. Studies of interactions between cells of blood vessel walls, such as endothelial and smooth muscle cells, with specific proteins such as those associated with platelets, blood coagulation, and fibrinolysis have been an area of only moderate research activity. The recent demonstration, however, that binding of thrombin to an endothelial binding site strikingly alters the enzymatic activity of thrombin has opened new directions. Concepts and technical methods appear to have so progressed that major advances should be possible in delineating the interactions between blood vessel wall moieties and hemostatic factors. The availability of targeted research funds to stimulate a substantial increase in research activity following the September 1982 conference is highly desirable.

The remarkable advances in the understanding of the biochemical reactions of hemostasis described earlier contrast strikingly with the meager progress in the understanding of production and catabolism of the hemostatic

factors. New techniques for culture of megakaryocytes make an important new tool available for studies of the control of platelet production. Yet, current activity is very modest and is not increasing as rapidly as anticipated. Similarly, the development of liver cell culture techniques opens opportunities for studies of the synthesis of the blood coagulation proteins. Again, only a very few laboratories appear to be involved in this area of research. Investigations in which these two culture techniques are applied to studies of the control of production of platelets and clotting factors would seem another important area for targeted research funds.

There have been no studies of the catabolism of the blood coagulation and fibrinolytic proteins. The reasons are not clear, but they may partly reflect an unfamiliarity of investigators with the concepts and techniques for research in this area. A workshop designed to educate the hemostasis community in the general area of mechanisms of plasma protein catabolism could well serve to stimulate research. Such a workshop also deserves a high priority.

Platelet Abnormalities and Disorders

As discussed earlier, platelets, or thrombocytes, are essential for normal hemostasis. When the concentration of platelets in the blood falls from its normal level of about 200,000 per microliter to below 30,000 per microliter, a very serious, potentially fatal bleeding tendency called severe thrombocytopenia is created. It results from one of two causes: a shutting off of the production of new platelets in the bone marrow or a very rapid destruction or loss of the platelets circulating in the blood. In addition, moderate types of thrombocytopenia, which results when the concentration of platelets is in the range of 50,000 to 100,000 per microliter, may be caused by a maldistribution of the circulating platelets secondary to their accumulation in an enlarged spleen.

Severe thrombocytopenia develops in a large number of clinical circumstances: in the leukemias; after bone marrow damage induced by drugs (such as chemotherapy for cancer), by radiation (such as overexposure to nuclear radiation), by antibodies directed against bone marrow cells, or by unknown causes; in patients with certain recognized immune disorders (such as systemic lupus erythematosus), in patients with drug reactions, and in otherwise apparently normal individuals in whom antibodies suddenly appear in the blood that attack the circulating platelets; as a complication of serious infections in which bacteria or

bacterial products gain access in large amounts to the bloodstream; and after massive bleeding from any cause (such as massive gastrointestinal hemorrhage from a duodenal ulcer) in which most of the patient's own blood must be rapidly replaced by transfused blood that does not contain viable platelets. Sudden, life-threatening thrombocytopenia can occur, along with hemolytic anemia and thrombi in the microcirculation, in an unusual catastrophic illness called thrombotic thrombocytopenic purpura (TTP).

In a different type of abnormality, patients can have normal numbers of platelets in their blood but yet bleed because the platelets do not function normally. Acquired platelet malfunction can be found in a number of common clinical circumstances, including: open heart surgery, uremia, myeloproliferative disorders, multiple myeloma, and high-dosage administration of some of the newer penicillins. In addition, a small group of hereditary disorders have been recognized in which patients have normal platelet counts but, because of platelet malfunction, have a lifelong tendency to bleed. Studies of why the platelets function abnormally in these unusual patients have substantially advanced the knowledge of normal mechanisms of platelet function in hemostasis and also in thrombotic diseases, which are the leading cause of death in the U.S. population.

Bleeding resulting from thrombocytopenia represents a major cause for morbidity and mortality in disorders impairing bone marrow function, such as the leukemias and aplastic anemia. Thrombocytopenia also contributes substantially to the morbidity of patients with other malignant disorders, frequently limiting the amount of chemotherapy that a patient may be given. Excessive blood loss from either thrombocytopenia or platelet dysfunction is a feared complication of open heart surgery, particularly coronary artery bypass surgery, and contributes substantially to the morbidity of the procedure. Bleeding secondary to acquired platelet abnormalities also complicates the management of the very large number of patients with end-stage renal disease currently maintained on hemodialysis programs. Thrombocytopenic bleeding is a frequent problem in intensive care units, particularly in the care of patients critically ill with bacterial infections. In chronic idiopathic thrombocytopenic purpura, a relatively common hematologic disorder, a previously completely normal adult suddenly develops a life-threatening bleeding tendency that may persist or recur over years.

Although rare, the quantitative platelet disorders that are hereditary represent important conditions requiring continued thorough investigation. As already mentioned, their study has yielded knowledge that is a key to an understanding of platelet function in normal hemostasis and in the thrombotic disorders. Thrombotic thrombocytopenic purpura similarly requires further intensive study. In this disorder, one or more disturbances in

the plasma cause a previously normal patient to form crops of platelet-fibrin thrombi in the small vessels of many organs. Discovering the cause or causes for this disorder should provide fundamental insight into the mechanisms maintaining the non-thrombogenic property of the normal endothelial inner lining of the blood vessels.

State of Knowledge in 1972

Effective test methods for the clinical evaluation of platelet function had been developed by 1972 and were available in some but by no means all university medical centers: the template bleeding time, platelet aggregometry techniques utilizing ADP, collagen, epinephrine, and thrombin, and a glass-bead platelet adhesion test. Methods for quantifying platelet-ADP release and platelet-serotonin uptake and release were primarily investigational as was the ^{51}Cr technique for measuring the intravascular life span of platelets.

Idiopathic thrombocytopenic purpura (ITP) was recognized as a disorder associated with an antibody in a patient's plasma that could cross the placental barrier and induce thrombocytopenia in the newborn infant. The antibody in the plasma was also known to induce transient thrombocytopenia when transfused into volunteers and also when stored and then transferred back into the patient after recovery. Techniques had been developed for detecting platelet antibody in plasma in vitro, but the techniques were difficult to standardize and not reproducible in different laboratories.

Posttransfusion purpura was also recognized as an antibody-induced thrombocytopenia in which a female patient who did not possess the common platelet antigen PlA^1 would be transfused with PlA^1 positive blood and then develop an antibody that somehow caused destruction of her own platelets. Thrombocytopenia in patients receiving quinidine or quinine was also known to be antibody-induced; an antibody to the drug was thought to react with the drug in the plasma. After the reaction, the drug-antibody complex attached itself to the platelet surface.

Acquired abnormalities of platelet function had been identified as a cause for bleeding in nonthrombocytopenic patients with myeloproliferative disorders, in patients undergoing open heart surgery, in patients with paraproteinemias, and in patients with uremia. The platelet dysfunction of uremia had been shown to result in prolonged bleeding, decreased platelet aggregation with collagen, epinephrine, and ADP, and decreased availability of platelet-phospholipid clotting activity. The cause for the platelet abnormality was recognized as extrinsic to the platelet, and it stemmed from a plasma material or materials that could be

removed by repeated dialysis. Urea, guanidine, succinic acid, and phenolic acids had each been proposed as the material in plasma responsible for the platelet dysfunction.

The hereditary bleeding disorders in which there is a normal platelet count accompanied by prolonged bleeding had been divided into: von Willebrand's disease, which was recognized as a plasma disorder; thrombasthenia, which is a severe bleeding disorder characterized by failure of clot retraction and failure of platelet aggregation with ADP; and the thrombocytopathies, which are milder bleeding disorders characterized by inadequate ADP release from activated platelets. The last had been divided into storage pool disease, in which the dense bodies of the platelets are deficient in ADP, and malfunctions of the release mechanism, in which the dense bodies contain adequate amounts of ADP but fail to release the ADP after platelet stimulation. Another rare hereditary platelet disorder had been recognized, Bernard-Soulier disease, in which very large platelets are present in the circulation. A defect in platelet function had been delineated in this disorder.

Aspirin was known to impair release of ADP from normal platelets stimulated by collagen or epinephrine. This effect was associated with slight prolongation of bleeding of most normal individuals but with striking prolongation of bleeding in occasional hypersensitive patients, in patients with von Willebrand's disease, and in patients with severe hemophilia. How aspirin impaired platelet-ADP release was unknown.

The clinical features of thrombotic thrombocytopenic purpura were well-known, but its pathogenesis was not known. Evidence existed, however, that it was not a clinical manifestation of the generalized Shwartzman reaction--namely, that the crops of thrombi did not result from disseminated intravascular clotting followed by deposition of fibrin in otherwise normal small vessels. A multitude of treatments had been proposed, including steroids, heparin, splenectomy, and infusions of dextran. A single report had appeared many years earlier of a patient who had survived after exchange transfusion.

Platelet concentrates were becoming increasingly available for use in the treatment of thrombocytopenic bleeding. Storage of platelets at 22°C rather than 4°C had been shown to prolong their viability. Evidence was beginning to be obtained that the use of platelets from HLA-compatable donors would delay isoimmunization and resultant loss of therapeutic effectiveness of the transfused platelets.

Program Goals Through 1982

Goals of research on platelet abnormalities and disorders, as stated in the 1972 National Program, were:

- Define mechanisms whereby platelets may be reduced in number or changed in function.
- Improve methods for detecting disorders of platelet function.
- Develop methods that correct or improve platelet function in various disorders.
- Determine the effect of drugs on platelet reactivity.

More specific long-term objectives of the DBDR that evolved over the past decade, partly from recommendations of the Blood Diseases and Resources Advisory Committee, were:

- Standardize routine methods for testing platelet function.
- Correlate the results of in vitro platelet function testing with in vivo platelet function.
- Define the biochemical defects of platelets in the hereditary platelet disorders, in uremia, and in liver disease.
- Isolate and characterize the platelet antigens that cause the development of platelet antibodies, and evaluate the clinical usefulness of the measurement of platelet antibodies in clinical management.

Accomplishments Through 1982

Advances in knowledge of normal platelet structure, metabolism, and responses to activation expanded the knowledge base necessary for understanding the causes of platelet dysfunction in the hereditary and acquired platelet disorders. In addition, technical accomplishments, particularly the development of methods for isolating and characterizing platelet membrane glycoproteins, made experiments possible that delineated the structural or enzymatic abnormalities responsible for platelet dysfunction in several of the hereditary disorders.

Major accomplishments directly related to specific platelet disorders can be summarized as follows:

Hereditary Qualitative Platelet Disorders

The defective platelet adhesion and impaired ristocetin-induced aggregation of platelets in the Bernard-Soulier syndrome were shown to result from absence of the platelet surface membrane glycoprotein, GP I. This finding provided the first evidence that GP I functions as the platelet receptor for the von Willebrand protein.

The absence of ADP-induced platelet aggregation, the failure of clot retraction, and the markedly diminished platelet fibrinogen of thrombasthenic platelets were found to result from the absence of or a deficiency in function of the platelet-surface glycoprotein complex GP II_b-III_a. This breakthrough provided strong evidence that the binding of fibrinogen to a receptor associated with the II_b-III_a complex is a key reaction of normal platelet cohesion.

One group of inherited abnormalities of the platelet release mechanism was found to result from impaired synthesis or function of thromboxane A_2. Hereditary cyclooxygenase deficiency and impaired sensitivity to thromboxane A_2 were delineated as specific hereditary disorders causing mild bleeding symptoms. The effect of aspirin upon platelet aggregation and ADP release was shown to stem from thromboxane A_2 synthesis that was impaired by inactivation of cyclooxygenase.

Additional hereditary disorders of platelet quality were discovered, including: the gray platelet syndrome, resulting from abnormal release of platelet alpha-granule contents; and a form of pseudo-von Willebrand's disease associated with abnormal affinity of platelets for von Willebrand protein.

Idiopathic Thrombocytopenic Purpura

The understanding of the pathogenesis and treatment of idiopathic thrombocytopenic purpura increased substantially. Reliable methods were developed to measure platelet-bound IgG, and elevated levels of platelet-bound IgG were found in the vast majority of ITP patients studied. Adrenal steroids were shown to diminish splenic sequestration of antibody-coated platelets, presumably by impeding macrophage phagocytic function. The spleen removed from patients with ITP was shown to synthesize platelet antibody. A milder variant of ITP was identified with these characteristics: evidence of platelet antibody, increased

platelet turnover, mild or no thrombocytopenia, functional abnormalities of platelets, and a mild tendency to bleed or bruise.

Thrombotic Thrombocytopenic Purpura

Plasma exchange was demonstrated to induce a remission in a high percentage of patients with thrombotic thrombocytopenic purpura. For the first time clinicians were provided with a therapy that can arrest the progress of this catastrophic illness. Investigators were provided with an important clue that thrombotic thrombocytopenic purpura stems from an abnormality of a plasma factor.

Additional accomplishments in the clinical management of patients included:

Standard Techniques for Assessing Platelet Function. Platelet function testing became widely available in major hospitals throughout the nation. A workshop and publication on platelet function tests supported by the DBDR contributed significantly to this accomplishment.

Methods for Clinical Assessment of Platelet Consumption. New methods include a platelet survival technique using radioactive indium, which simplifies methodology and makes external imaging feasible, and a nonisotopic method for evaluating platelet survival that can be used in the pregnant patient. Radioimmunoassays for PF4 and beta-thromboglobulin were found valuable in evaluating whether different thrombocytopenias result primarily from intravascular or extravascular platelet destruction.

Replacement Therapy. Platelet concentrates became generally available for replacement therapy. This advance had a tremendous impact on cancer chemotherapy and on bone marrow transplant programs. Methods to forestall or delay isoimmunization were improved, including the widespread use of HLA-matched platelets and the design of procedures to obtain large numbers of platelets from single donors.

State of Knowledge in 1982

Several hereditary disorders of platelet function can now be characterized in terms of specific structural defects or enzymatic defects of platelets. Increasingly precise methods, such as the use of monoclonal antibodies to different epitopes of a platelet-surface glycoprotein, are becoming available that will permit further detailed characterization of these defects at the molecular level. Application of these methods should not only result in the recognition of yet undefined hereditary disorders of

platelets, but also increase the general understanding of aberrant platelet function, including platelet disorders in the thromboembolic diseases.

Advances in the understanding of the pathogenesis and natural history of adult idiopathic thrombocytopenic purpura have resulted in long-term therapeutic approaches that reduce the risks of bleeding and of extensive therapy. The availability of reproducible, simplified methods for measuring platelet-bound IgG should permit a definitive evaluation of the use of this test in diagnosis and clinical management. Most importantly, concepts and techniques have advanced to where rapid progress should be possible in delineating the specific platelet antigens against which platelet antibodies in this disorder are directed. Such data, essential for understanding the pathogenesis of ITP, may also permit design of treatment based upon reestablishing immune tolerance to the platelet antigens involved. It should also be possible to determine whether patients with antibodies against different platelet antigens have different manifestations of disease and different long-term prognoses.

Although investigators agree that plasma exchange induces remissions in most patients with thrombotic thrombocytopenic purpura, they disagree as to whether this remission results from removing a deleterious material in the patient's plasma or from supplying a material in the plasma given to the patient. Different groups have used different regimens of plasma removal and replacement. Some groups have reported remissions in patients given plasma transfusions without plasma removal. At least two groups, however, have reported that the plasma of patients with the disease contains material that injures endothelial cells in culture, as measured by ^{51}Cr release from the cells. Other investigators have reported that the plasma contains a platelet-activating factor that causes platelet aggregation, and still other investigators believe that a reduced production of prostacyclin leads to the formation of the multiple, small, bland thrombi that are characteristic of the disorder. Although an important advance has been made in the clinical management of the disease, its pathogenesis, with the implications for understanding the processes that normally prevent thrombi from forming in the microcirculation, remains unknown.

Numerous abnormalities of platelets have now been described in the myeloproliferative disorders, including absent platelet aggregation with epinephrine, reduced platelet-phospholipid clotting activity (PF3), loss of epinephrine receptors, loss of receptors of PGD_2, and impaired oxidation of arachidonic acid by way of the lipoxygenase pathway. It has not yet been possible, however, to relate specific defects to either the abnormal tendency to bleed or the abnormal tendency for thrombosis.

Little more is known today than in 1972 about the cause or causes of abnormal platelet function in uremia. The dialyzable "middle molecules" responsible for the functional defects and prolonged bleeding have not yet been identified. Production of prostacyclin by vessel segments was recently reported to be increased in uremic patients. Recently, administration of cryoprecipitate has been recommended for control of uremic bleeding. The mechanism of its action is unknown.

Transfusion of platelet concentrates has become standard therapy for the control of bleeding in patients with thrombocytopenia secondary to decreased platelet production. Platelet concentrates are also given prophylactically to such patients, but the indications, benefits, and deleterious effects of prophylactic platelet transfusions have not been rigorously established. Methods of preparation and liquid storage of platelets have been standardized. Frozen platelets have been used successfully in investigational studies. Freezing might prove particularly valuable as a means of storage of a patient's own platelets for subsequent reinfusion to prevent thrombocytopenic bleeding after cancer chemotherapy. Simple and practical methods to detect immunization to platelet antigens and to perform pretransfusion cross-match are not yet available.

Program Goals 1982 to 1987

The broadly stated goals described for the period through 1982 remain valid for the period 1982 to 1987. In addition, the following specific goals are defined:

- Continue intensive study of the defects responsible for each of the rare hereditary disorders of platelet function in order to characterize, at the molecular level, the platelet structural components and metabolic events required for platelet function in normal hemostasis and affecting platelet participation in the pathogenesis of thrombosis.

- Characterize the platelet antigens and the antigenic specificity of the antibodies arising in patients with idiopathic thrombocytopenic purpura.

- Elucidate the pathogenetic mechanism or mechanisms for thrombotic thrombocytopenic purpura and related syndromes.

- Delineate the pathogenesis and management of bleeding in uremia, including the identification of the dialyzable material or materials that impair platelet function, the determination of their mechanism of action, and the

further evaluation of the reported therapeutic effectiveness of infusion of cryoprecipitate.

- Improve platelet transfusion therapy through clarification of indications, development of better methods of preparation and storage of platelet concentrates, development of methods to prevent and to detect immunization to platelet antigens and contaminating lymphocytes, and development of methods to perform pretransfusion cross-match.

Research Activities 1982 to 1987

Current Activities

Many of the 70 projects involving platelets discussed under normal hemostasis can equally well be viewed as part of the DBDR program on platelet disorders and abnormalities. In addition to the projects on normal hemostasis, the Division supports 25 projects that deal specifically with one or more aspects of platelet disorders.

Investigations in the qualitative platelet disorders related to the mechanisms of platelet dysfunction are receiving substantial support. In the hereditary qualitative platelet disorders, platelet surface membrane glycoproteins are being isolated and characterized in relation to abnormal platelet function. Studies of the platelet surface glycoprotein defect in Glanzmann's thrombasthenia have already led to a landmark advance in understanding the mechanism of platelet aggregation, and such studies continue to receive emphasis. In other investigations, abnormalities of platelet granule content and of platelet secretion in disorders of platelet function are being related to clinical manifestations resulting from these abnormalities and also to the roles of granule materials and platelet secretion in normal platelet function. Abnormalities of platelet lipid metabolism in platelet functional disorders are also under investigation.

Studies of mechanisms of injury to platelets in those thrombocytopenias stemming from immune platelet destruction are in progress in several laboratories. These include studies of the interaction of IgG complexes with Fc receptors of platelets, of platelet receptors for drug-antibody complexes, and of the effect of complement binding and activation upon platelets. Clinical investigations are also being supported, including a major study, involving three of the four Specialized Centers of Research (SCOR's) on thrombosis, of the incidence, mechanism, and clinical consequences of heparin-induced thrombocytopenia.

The most common hereditary disorder affecting platelet hemostatic function, von Willebrand's disease, stems from a deficiency of a plasma component, the von Willebrand protein, which is necessary for platelet adherence to subendothelium. Current activities related to von Willebrand's disease are discussed later.

New Activities

Thrombotic Thrombocytopenic Purpura. Despite interest stimulated by the effectiveness of plasma exchange in thrombotic thrombocytopenic purpura, research directed to identifying the plasma abnormality or abnormalities in this disease is limited. Clincial investigations are limited by the infrequent, sporadic nature of the disease; no single group sees enough patients to undertake extensive clinical investigations of therapy. Therefore, the possibility of establishing a thrombotic thrombocytopenic purpura study group deserves serious consideration. Such a group might address several important clinical questions, including:

Is thrombotic thrombocytopenic purpura one or several diseases?

Can criteria for diagnosis be established for subgroups of thrombotic thrombocytopenic purpura?

Can clinical manifestations on presentation be related to the need for different schedules of plasma exchange?

What is the natural history of this disorder, or disorders?

In addition, the members of the group could undertake to investigate jointly the nature of the plasma abnormality or abnormalities by assuming responsibility for the evaluation of different possibilities. Collection and storage of plasma from patients would be standardized, and plasma from each patient would be distributed to all members of the group.

Uremic Bleeding. Uremic bleeding remains a major problem in the clinical management of the very large number of patients with end-stage renal disease who are being maintained on hemodialysis or chronic peritoneal dialysis. Studies are needed to correlate correction of the time of a patient's prolonged bleeding with changes in readily measurable markers in the plasma. Although it has been known for years that uremic plasma contains dialyzable materials that impair platelet function, their identity remains unknown. A postdialysis plasma profile needs to be delineated that could be used to determine whether dialysis has adequately

removed the materials interfering with platelet function. Moreover, chronic peritoneal dialysis needs to be compared with hemodialysis in its effects upon the length of time of bleeding. In addition, the infusion of cryoprecipitate in the management of uremic bleeding needs further evaluation. The limited progress in understanding uremic bleeding may partly reflect the specific clinical characteristics of the questions that need answering. Support by the contract mechanism may be more appropriate for such work than support by investigator-initiated research.

Platelet Concentrates for Transfusion Therapy. The new activities needed to improve platelet concentrate transfusion therapy are described in detail in section 5 of this report ("Blood Resources").

Hemophilia and Other Bleeding Disorders

The hemophilias and von Willebrand's disease are the most common, and therefore the most important, of the hereditary disorders in which one or another of the proteins of the blood required for the control of bleeding after injury or surgery either is missing from the blood or is present in an abnormal, nonfunctional form. Affected individuals may have major hemorrhages throughout their lifetime. Because of sex-linked genetic transmission, the hemophilias affect only males. Von Willebrand's disease affects both males and females.

The hemophilic population of the United States has been estimated at between 10,000 and 25,000. The disease requires lifetime treatment at a cost that severely stresses the health care system. For example, the cost of using activated factor IX concentrates to bring a single episode of life-threatening bleeding under control in a hemophilic patient who has developed an inhibitor may exceed $50,000, and at times, even $100,000. Second, the preparation of enough factor VIII for the treatment of hemophilia represents a very heavy, steadily growing demand that threatens to exceed the capacity of the nation's blood resources.

Acquired bleeding disorders can develop in previously normal individuals as a complication of a large number of clinical conditions affecting function of the blood platelets, the blood coagulation reactions, the fibrinolytic reactions, or combinations of these processes. Conditions in which bleeding may occur primarily because of abnormalities of the blood coagulation factors include: disorders compromising liver-cell function, such as hepatitis or cirrhosis of the liver; disorders interfering with vitamin K metabolism, such as those of the small intestine; autoimmune disorders in which antibodies to blood coagulation

proteins are formed; and certain malignancies, head trauma, and complications of pregnancy and delivery, in which disseminated intravascular coagulation may deplete the blood of coagulation proteins.

Acquired bleeding disorders represent a major cause of morbidity and death in a broad spectrum of illnesses in which patients may begin to bleed seriously with little warning. Of particular concern is the severe gastrointestinal bleeding that is a feared complication of liver disease and a major cause of death in cirrhosis of the liver. Hemorrhage, which is difficult to stop because of failure of hemostasis, may be an important factor in the morbidity and mortality associated with some forms of severe trauma. Although not common, unexpected, profuse bleeding occasionally develops as a catastrophic complication of pregnancy and delivery, endangering the life of both the mother and the infant.

State of Knowledge in 1972

Hemophilia and von Willebrand's Disease

Hemophilia was known to be two diseases: factor VIII deficiency (hemophilia A), which accounted for 80 percent of patients, and factor IX deficiency (hemophilia B), which accounted for the remainder. Factor VIII deficiency was also known to exhibit genetic heterogeneity, with about 90 percent of patients having no or trace amounts of circulating factor VIII antigen and 10 percent of patients having substantial amounts of circulating factor VIII molecules that lacked normal coagulant activity. Factor IX deficiency was also known to exhibit genetic heterogeneity: three variants, which differed in their content of factor IX antigen and their effect upon the clotting of plasma with ox brain tissue factor, had been delineated.

Von Willebrand's disease was recognized as a disorder in which a plasma protein of very high molecular weight was present either in a reduced amount or in an altered form. This protein was known to be required for the normal formation of platelet hemostatic plugs after vessel-wall injury. Von Willebrand protein was also known to be required to maintain a normal level of factor VIII coagulant activity in plasma. Infusion of von Willebrand protein into patients with von Willebrand's disease had been shown to cause endogenous plasma factor VIII coagulant activity to increase and to be maintained for many hours thereafter.

The association between factor VIII coagulant activity and von Willebrand protein was unknown; a smaller protein with factor

VIII coagulant activity could be separated from von Willebrand preparations. It was not known whether the smaller protein represented a subunit of the von Willebrand protein or a separate factor VIII coagulant protein that circulates in plasma as a complex with the von Willebrand protein.

Factor IX was known to be a vitamin K-dependent protein that during clotting is activated by proteolytic cleavage. Factor XI_a was the only recognized activator of factor IX. Factor IX had not been purified, and virtually nothing was known about its structural properties.

Exposure to trace amounts of thrombin had been shown not only to increase strikingly the coagulant activity of factor VIII but also to result, in minutes, in the deterioration of the coagulant activity. Activated factor IX, thrombin-activated factor VIII, phospholipid, and calcium ions were known to form a complex that functions as an activator of factor X. Activated factor IX was recognized as the enzyme responsible for factor X activation, in which factor VIII is a cofactor.

Factor IX was known to be synthesized in the liver. The site of synthesis of von Willebrand factor and of factor VIII coagulant protein were unknown. Factor VIII was known to have a short intravascular half-life of about 12 hours, and factor IX to have a somewhat longer half-life.

Assays for factor VIII and factor IX coagulant activity were available in coagulation research laboratories and in most large university medical centers but were not generally available in major community hospitals. The technique of electroimmunoassay for von Willebrand antigen in plasma had been developed but was available in only a few research laboratories for the diagnosis of von Willebrand's disease. The observation had just been made that the antibiotic ristocetin failed to aggregate platelets in platelet-rich plasma from patients with von Willebrand's disease.

Factor VIII and factor IX coagulant assays were being used in large centers for the detection of hemophilia carriers. It was recognized that detection of the hemophilia A carrier could be improved by the combined use of a factor VIII coagulant assay and an electroimmunoassay for von Willebrand factor, but the latter technique was available in only a few research laboratories.

Factor VIII concentrates of two types were available and in wide use: frozen, single-donor cryoprecipitate units, and commercial, freeze-dried concentrates prepared from pooled plasma. A factor IX concentrate, prepared from pooled plasma, was also available. It had just been recognized that such concentrates

often contain activated materials that can cause thrombosis in patients receiving large amounts of factor IX concentrate. The very high risk of transmitting hepatitis through the use of pooled concentrates was appreciated.

Home therapy for hemophilia was under evaluation in a few but growing number of institutions. In clinical trials in England, antifibrinolytic agents had just been shown to reduce substantially the amount of factor VIII replacement therapy needed after dental procedures. Factor VIII inhibitors were reported to develop after replacement therapy in an estimated 5 to 20 percent of patients with hemophilia A. This complication was recognized as a major, unsolved problem in the care of patients with hemophilia A. A single report had appeared of the use of an activated factor IX concentrate to treat bleeding in the patient with a factor VIII inhibitor. The use of aspirin had been shown to increase the tendency of the hemophilic patient to bleed, as demonstrated by marked prolongation of bleeding.

Acquired Bleeding Disorders

Vitamin K-deficiency was known to depress factor VII, factor IX, factor X, and prothrombin coagulant activity, but the mechanism of this action was unknown. Liver disease was known to suppress the synthesis of most of the blood coagulation proteins and to enhance fibrinolytic potential. Liver disease was also thought to predispose to intravascular coagulation that could further decrease levels of clotting factor. The extent to which each of these factors is involved in the uncontrollable gastrointestinal bleeding of patients with advanced cirrhosis was not clear.

Acquired inhibitors of blood coagulation were being increasingly recognized as a cause of hemostatic failure. Factor VIII inhibitors had been shown to be antibodies of the IgG class and to occur not only as a complication of transfusion therapy in hemophilia but also apparently spontaneously in occasional nonhemophilic patients. A few patients had been discovered, primarily postoperatively, with acquired inhibitors to factor V. The lupus anticoagulant phenomena had been well described not only in patients with systemic lupus erythematosus but also in patients with many other disorders.

Bleeding secondary to intravascular coagulation, with consumption of clotting factors and secondary fibrinolysis, was recognized as a complication of a variety of infections, of pregnancy and delivery, of malignancy, and of certain types of tissue trauma. Gram-negative endotoxin entering the blood stream was known to trigger intravascular coagulation, but the mechanism

was debated. Some thought it resulted from a reaction between endotoxin and white blood cells, whereas others thought that damage to platelets and activation of factor XII were the triggering mechanisms.

Program Goals Through 1982

Hemophilia and von Willebrand's Disease

The broad goals through 1982, as stated in the 1972 National Program and the Fifth Report of the Director, 1978, were:

- Develop a better understanding of the genetic and pathologic mechanisms underlying hemophilia and other bleeding disorders in order to develop improved diagnostic techniques and specific treatments.

- Develop improved techniques to harvest, concentrate, and store factors VIII and IX so they can be made available, at a reasonable cost, to all hemophiliacs who need them.

- Educate the public so that hemophilia carriers and patients can make informed decisions regarding their own lives.

More specific goals that evolved over the past decade, partly from recommendations of the Blood Diseases and Resources Advisory Committee, were:

- Isolate and characterize the components of the von Willebrand factor VIII complex related to procoagulant, antigenic, and von Willebrand factor activity.

- Determine the prevalence and genetics of the different von Willebrand syndromes, and develop new tests to identify asymptomatic carriers of von Willebrand's disease and of hemophilia.

- Elucidate the functional, immunological, and physicochemical characteristics of factor IX and their reaction to the abnormal coagulant function of the molecule in hemophilia B.

- Examine possible relationships between reduced levels of von Willebrand factor, as found in patients and animals with von Willebrand's disease, and the pathogenesis of atherosclerosis.

- Investigate the molecular mechanisms for the occurrence of factor VIII inhibitors in hemophiliacs and the natural history of the occurrence.

- Clarify the role of prothrombin complex concentrates in the treatment of patients with factor VIII inhibitors, and identify the procoagulant materials present in these concentrates.

- Develop the capability for the prenatal diagnosis of hemophilia and other hemorrhagic diseases.

- Evaluate the effects of modern treatment for hemophilia.

Acquired Bleeding Disorders

- Develop better methods for identifying and detecting individuals at risk for developing acquired bleeding disorders, through:

  - increasing the understanding of the pathogenetic mechanisms responsible for the different acquired bleeding disorders.

  - improving diagnostic methods for characterizing the specific defects present in the different acquired bleeding disorders.

- Improve specific treatment methods for the acquired bleeding disorders.

Accomplishments Through 1982

Hemophilia and von Willebrand's Disease

Much has been learned in the past 10 years about the physiochemical properties of factor VIII, the von Willebrand protein, and factor IX. After some years of differing opinions, it was established that the factor VIII coagulant protein and the von Willebrand molecule are separate molecule entities despite their association in plasma. Moreover, the von Willebrand molecule was found to exist in many forms, made up of multimers having molecular weight of 800,000 to 14,000,000. Variants of von Willebrand's disease were identified in which the total plasma concentration of von Willebrand protein was normal but the larger multimers were reduced in concentration. The von Willebrand molecule was also shown to contain carbohydrate, and the von

Willebrand molecule from some individuals with von Willebrand's disease was reported to possess abnormalities of carbohydrate structure.

After many years of little progress, the recent availability of monoclonal antibodies has made rapid advances possible in the purification and characterization of the factor VIII coagulant protein. Native coagulant factor VIII, however, still remains to be well characterized.

Factor IX has been purified from both bovine and human plasma. Its basic structure has been elucidated, the proteolytic cleavages responsible for its activation have been identified, and the partial amino acid sequence of bovine factor IX has been determined. At least three abnormal factor IX molecules have been purified from the plasma of patients with variants of hemophilia B in which the factor IX molecules have a reduced coagulant activity. The properties of these variant molecules have been partially characterized.

These accomplishments in biochemistry have led to a growing understanding of the molecular mechanisms involved in the pathogenesis of several forms of hemophilia and von Willebrand's disease. Accomplishments in physiology have been less dramatic. A major step, however, has been the identification of the endothelial cell as the site of synthesis of the von Willebrand protein. It has also been established that both von Willebrand factor and factor VIII coagulant protein are acute phase proteins whose plasma concentrations rise in inflammatory states, after surgery, after severe exercise, and after other stressful situations. This observation sheds new light on the interpretation of measurements of factor VIII activity and von Willebrand antigen in clinical disorders.

Techniques were developed during the decade to study adhesion of platelets in circulating blood to exposed vascular subendothelium. The importance of von Willebrand factor for adhesion was shown to vary with wall shear stress. Von Willebrand protein was also identified as a normal constituent of vascular subendothelium. Animal models of von Willebrand's disease were discovered and used to study whether von Willebrand factor-mediated platelet adhesion was a significant variable in the pathogenesis of atherosclerosis.

Methods to diagnose von Willebrand's disease improved strikingly over the past 10 years. With the technique of ristocetin-induced platelet aggregation, a quantitative assay for von Willebrand factor in plasma was developed. The technique of electroimmunoassay of von Willebrand antigen in plasma became clinically applicable. Availability of these two tests led to the

discovery of many patients with milder von Willebrand's disease and the realization that this disorder is the most common of the hereditary bleeding diseases.

Crossed immunoelectrophoresis of von Willebrand factor in plasma was developed as a clinical test for the recognition of the variant form of von Willebrand's disease in which antigen levels are normal but the larger von Willebrand molecules are missing or are fewer. More sensitive techniques for characterizing the multimers of von Willebrand factor were developed, and research that makes use of the techniques promises a further understanding of the functions of this key protein in hemostasis.

A radioimmunoassay was developed for the factor VIII coagulant protein (VIII:Cag). Its use with the coagulant factor VIII assay has increased the accuracy of carrier detection in hemophilia A to the 85 to 95 percentile range. Measurement of VIII:Cag and factor VIII coagulant activity in fetal blood, obtained by fetoscopy, has permitted in utero detection of a fetus with hemophilia A. The risks of bleeding after blood sampling, however, and the need for personnel with very specialized skills for taking samples require the continued search for a safer and more widely applicable method for in utero detection of hemophilia.

The clinical management of patients with hemophilia and related disorders has advanced remarkably over the past 10 years. A network of comprehensive care programs has been developed to deliver a full range of services. In addition, a cooperative hemophilia study group was formed to study the natural history of the disease, the incidence and patterns of behavior of inhibitors of clotting factors, and the effectiveness of currently available factor IX concentrates in ameliorating bleeding in the joints of patients with a factor VIII inhibitor. Administration of an antifibrinolytic agent became standard management for dental patients with hemophilia A and von Willebrand's disease. After clinical evaluations, home care programs became widespread. The ability of the patient or of a family member to infuse the patient with factor VIII at the first evidence of bleeding was shown to reduce days lost from school or work. Many patients have now become productive citizens.

Factor VIII inhibitor was found to occur in 10 to 15 percent of patients with hemophilia A. Two categories of these patients were delineated by the cooperative study group. A small group of patients was defined who responded satisfactorily to factor VIII infusion therapy in spite of low titers of factor VIII inhibitor. A large group of patients was defined who developed a sharp anamnestic immune response to treatment with factor VIII. The response indicated very high titers of factor VIII inhibitor.

These patients proved difficult to treat during episodes of bleeding despite the availability of both standard and "activated" factor IX concentrates. Immunosuppressive drugs were found unable to prevent the anamnestic response to factor VIII inhibitor in this larger group of patients.

The hemophilia cooperative study group also determined the use and demand for blood products and defined factor VIII requirements in relation to the severity of disease and inhibitor status. Average annual use of factor VIII per hemophiliac in the study was 40,000 units. It was calculated that approximately 70,000 units of factor VIII are used per surgical intervention. These studies provide a basis for future projections of needs.

With regard to chronic liver disease in hemophilic patients, the cooperative hemophilia study group delineated the incidence of hepatitis markers and abnormalities of liver function in its patient population. The characteristics of their liver disease were revealed by a study of liver histopathology in patients who for as long as they live receive transfusions of blood products.

The NHLBI also supported a major effort in hemophilia education. A fact book, an educational movie, and a slide series were prepared, evaluated, and found to be valuable additional tools for public education. The NHLBI jointly with the New York Academy of Science sponsored an interdisciplinary program on hemophilia and von Willebrand's disease.

Acquired Bleeding Disorders

Simple methods were developed for measuring the coagulant activity of each of the blood coagulation factors. These methods were adopted by the clinical laboratories of most university and many community centers, with resultant substantial improvement in the diagnosis and management of patients with acquired bleeding disorders.

New assay methods were designed that were based upon the use of chromogenic or fluorogenic substrates specific for the esterolytic activity of individually activated coagulation proteins. These techniques allow for a major innovation in research methods, and they have been used in a variety of studies, including investigations of the pathogenesis of the acquired bleeding disorders. They are also beginning to be used in clinical laboratories.

Intravascular coagulation after exposure of blood to endotoxin was shown to be associated with the development, by the

blood monocyte, of tissue factor activity. This observation stimulated a resurgence of interest in the roles of cellular procoagulant activities in blood coagulation.

Vitamin K-deficiency was identified as a surprisingly common cause for acquired bleeding in the very ill patient who is being maintained with intravenous fluids and is receiving a broad spectrum of antibiotics. Defibrination was identified as a common event that leads to abnormal bleeding after a head injury in which there is destruction of brain tissue. Intravascular clotting was not found to represent a major cause for a decrease in amounts of clotting factor in decompensated patients with severe hepatocellular liver disease. The use of factor IX concentrates, however, to treat bleeding in patients with liver disease was discovered to result in both intravascular coagulation and increased fibrinolysis and is therefore presently avoided. In contrast to the hemophilic patient, the nonhemophilic patient who develops a spontaneous factor VIII inhibitor often benefits from immunosuppressive therapy.

State of Knowledge in 1982

Hemophilia and von Willebrand's Disease

The accomplishments cited above have yielded a fundamental new understanding of the structure of the von Willebrand molecule, of the factor VIII coagulant protein, and of the relation between the two. Availability of monoclonal antibodies to both proteins permits the development of new techniques for their purification. The generation of a collection of monoclonal antibodies for epitopes on different segments of both molecules provides valuable new tools for examining structure-function relationships.

Knowledge of the activation of the factor VIII coagulant protein during hemostasis is still limited. The marked increase in activity of factor VIII after its exposure to trace amounts of thrombin in vitro has been repeatedly demonstrated. It is still not known, however, whether such activation of factor VIII by thrombin is a prerequisite for the effective participation of factor VIII in hemostasis. It is also now known that physiological amounts of thrombin, in the presence of a thrombin receptor on endothelial cells that modifies the activity of thrombin, activates the newly discovered vitamin K-dependent protein, protein C. Activated protein C, in turn, rapidly inhibits the coagulant activity of thrombin-activated factor VIII.

Methods for the purification of factor IX from normal plasma have been simplified. Techniques have also been developed for the purification of abnormal factor IX proteins in some variants of

hemophilia B. For studying the activation of normal and abnormal factor IX molecules, these important methods have been designed: computerized techniques for integrating peaks of activity on radioactivity profiles of gels, and an activation assay based upon the release of ^{3}H-labeled activation peptide. Monoclonal antibodies to factor IX have been generated and are being used in detailed structure-function studies of normal factor IX. Microtechniques for amino acid sequencing should permit the determination of the primary amino acid sequences of the variant factor IX molecules found in hemophilia B. The effects of binding calcium ions to normal factor IX upon the three-dimensional structure of factor IX are being elucidated.

The identification of a complex of tissue factor and factor VII_a as a major activator of factor XI is clarifying the mechanisms that activate factor IX. Kallikrein has also been identified as a nonphysiological activator of factor IX that could be important in the activation of factor IX in the preparation of concentrates of prothrombin complex. Techniques of preparation, activation, and separation of platelets have been developed that make it feasible to study the presumed generation of a complex of factor $VIII_a$ and factor IX_a on the platelet surface.

With the purification of von Willebrand protein, factor VIII coagulant protein, and factor IX protein, techniques for the isolation of DNA developed by molecular biologists will be applicable to the isolation of the DNA for these molecules. Identification and characterization of these DNA sequences may lead to recognition of abnormal DNA sequences in hemophilias and in von Willebrand's disease. Such information could form the basis of new techniques to diagnose the carrier state and the affected fetus.

The remarkable progress in recombinant DNA technology, which has led to the synthesis of such biologic materials as insulin in bacteria, makes the possibility of bacterial synthesis of factor VIII or factor IX for clinical use more real than it was when it was first suggested in the 1972 National Program; but many problems related to the need for posttranscriptional modifications of the molecules, such as addition of carboxy groups to form gamma-carboxyglutamic acid molecules in factor IX, can be foreseen.

Despite the remarkable progress of the past 10 years, important problems remain unsolved. The fundamental question of the cellular site of synthesis of factor VIII coagulant protein remains unanswered. The mechanisms responsible for the short intravascular half-life of 12 hours for factor VIII are unknown. Why plasma factor VIII coagulant activity cannot be maintained in the absence of the von Willebrand molecule also remains a mystery. The role of protein C in the physiological regulation of factor VIII catabolism remains to be explored.

Although much has been learned about the incidence and natural history of inhibitors of factor VIII, what determines whether a patient will develop an inhibitor following transfusion of exogenous factor VIII remains a fundamental unanswered question. Developments in immunology have clarified mechanisms by which antigen is processed and the synthesis of antibody is regulated. Such knowledge may now be applicable to studies of the mechanisms by which inhibitor in hemophilia A is formed. The development of monoclonal antibodies may also now make it possible to identify allotypes of human factor VIII coagulant protein and to examine their possible function in the generation of inhibitors.

Although clinical studies suggest that both nonactivated and activated prothrombin complex concentrates can be useful in the management of bleeding in patients with inhibitors, the substance or substances and the amounts in the concentrates responsible for this therapeutic effect have yet to be identified definitively. Moreover, the properties responsible for the thrombotic potential of concentrates have not yet been established. Animal models have been developed that should facilitate studies of thrombotic properties in concentrates.

A chromatographic technique, which utilizes insolubilized monoclonal antibody to factor V, was developed to purify factor V from human plasma. This advance raises the possibility that insolubilized monoclonal antibodies can be used to develop better methods for preparing factor VIII and factor IX concentrates for clinical use.

Acquired Bleeding Disorders

New information about the blood coagulation reactions, such as their modulation by protein C_a, and major advances in understanding fibrinolytic mechanisms, such as the enhanced affinity of vascular activator for plasminogen in the presence of fibrin, provide new theoretical bases for the design of studies of the pathogenesis, manifestations, and management of the acquired bleeding disorders. New tools--chromogenic assays, immunoassays for coagulation proteins and their activation products, assays for complexes of activated hemostatic factors and protease inhibitors--make such studies feasible.

Thus, the design of immunologic assays for the newly discovered vitamin K-dependent proteins C and S make it possible to study the kinetics of their production and catabolism in disorders affecting vitamin K metabolism. Immunologic assays for fibrinopeptide A and for cleavage products of prothrombin provide new, sensitive methods for monitoring intravascular coagulation in complications of pregnancy and delivery and in patients with

malignant disease. New tests for evaluating fibrinolysis, such as measurement of alpha-2-antiplasmin activity and plasmin-antiplasmin complexes, should permit a clearer evaluation of the possible role of excessive fibrinolytic activity in the uncontrollable bleeding that is a major cause of death in chronic liver disease.

Autoantibodies to the plasma proteins that are involved in hemostasis continue to be identified as rare but important causes for severe acquired bleeding disorders. They include acquired factor VIII inhibitors in the postpartum state, antibodies to von Willebrand protein in patients with macroglobulinemia of Waldenstrom, and antibodies that bind prothrombin but do not neutralize its in vitro coagulant activity in patients with acquired hypoprothrombinemia-lupus inhibitor syndrome. Assay systems have been designed to measure precisely and accurately the levels of plasma autoantibodies in these disorders. Studies of such patients and of the effect of immunosuppressive therapy upon their progress may provide unique opportunities for observations that are generally applicable to the cause of the loss and reestablishment of tolerance to normally occurring proteins.

Program Goals 1982 to 1987

The specific goals that gradually evolved over the past decade from the broad goals enunciated in 1972 may be further modified and extended for the period 1982 to 1987, as follows:

Hemophilia and von Willebrand's Disease

- Purify and characterize structure-function relations of coagulant factor VIII, von Willebrand protein, and the abnormal variants of coagulant factor VIII and von Willebrand protein found in some forms of hemophilia A and von Willebrand's disease.

- Continue studies of the structure-function relation of normal factor IX and of abnormal factor IX variants found in hemophilia B. Characterize the structural basis for the impaired coagulant function of abnormal factor IX variants.

- Study the hemostatic functions of von Willebrand factor in relation to platelet function, maintenance of plasma coagulant factor VIII activity, preservation of normal vascular wall integrity, and effect upon vascular responses to injury in the pathogenesis of atherosclerosis.

- Study at the molecular level the activation and interaction of factor VIII, factor IX, and platelets during blood coagulation in relation to the cause for the unique severity of bleeding in patients with hemophilia A or hemophilia B.

- Elucidate the factors regulating the synthesis and catabolism of von Willebrand protein, factor IX, and coagulant factor VIII. Identify the site of synthesis of coagulant factor VIII.

- Identify and characterize the genes for von Willebrand protein, factor IX, and coagulant factor VIII. Explore the use of recombinant DNA techniques to clone these genes. Apply techniques of genome analysis of cultured amniotic tissue fluid cells to the problem of identifying hemophilia and the carrier state in utero.

- Further characterize the genetics and molecular abnormalities of the variant forms of von Willebrand's disease and the design of simple test methods for their clinical diagnosis. Standardize techniques for monitoring replacement therapy of bleeding in von Willebrand's disease. Evaluate therapeutic roles of materials stimulating synthesis or release of the von Willebrand molecule, such as 1-desamino-8-d-argining vasopressin.

- Identify the immunologic mechanisms responsible for the formation of factor VIII antibodies in 15 percent of patients with hemophilia A. Develop techniques to prevent formation of antibodies and to regain tolerance to factor VIII in patients with antibodies.

- Develop improved factor VIII and factor IX concentrates for replacement therapy, including a factor IX concentrate without thrombogenic properties. Characterize the materials in present prothrombin complex concentrates responsible for thrombogenesis and for control of bleeding in the factor VIII inhibitor patient.

- Continue to evaluate the techniques and results of modern management of hemophilia: standardization of optimal factor VIII dosage schedules for supervised self-treatment, documentation of the course of chronic liver disease, and further exploration of the clinical consequences of circulating immune complexes.

- Produce and develop methods of distribution of concentrates for use in uncommon hereditary hemorrhagic disorders, such as in factor XI deficiency, where

commercial preparation of concentrates is not economically feasible.

Acquired Bleeding Disorders

- Apply new knowledge of the regulation of blood coagulation and fibrinolysis, and new, more sensitive and specific test methods to studies of the pathogenesis, manifestations, and therapy of the different bleeding disorders.

- Explore relationships between the acquired hemostatic defects arising in different disease states and manifestations of disease other than bleeding, such as alterations in inflammatory and immune processes, and the growth of tumor metastases.

- Develop improved replacement products for the management of bleeding in acquired bleeding disorders.

Research Activities 1982 to 1987

Hemophilia and von Willebrand's Disease

Current Activities. Studies are currently in progress in a number of laboratories on the purification and characterization of the von Willebrand molecule and the coagulant factor VIII molecule and of the interaction of the von Willebrand molecule with platelets. On a smaller scale, studies are being supported of the properties and function of normal factor IX and, to a limited degree, of the properties of variant factor IX molecules in hemophilia B. Four laboratories are working with animal models of hemophilia A, von Willebrand's disease, or both; studies exploring the relation between von Willebrand factor and the pathogenesis of arterial wall injury are in progress in at least two laboratories.

Work to delineate the genes for the von Willebrand molecule and the coagulant factor VIII molecule has begun. A study of endothelial cell and megakaryocyte synthesis of von Willebrand factor is being supported. In several laboratories, immunologic assays for coagulant factor VIII antigen are being evaluated for the diagnosis of the hemophilia carrier state, and in one laboratory, for the in utero diagnosis of hemophilia A. The chemical modification of factor VIII and factor IX to increase in vivo half-life is being evaluated.

Current activities hold promise in the near future for delineating the basic subunit structure of the von Willebrand molecule, the molecular mechanisms for multimer formation, and the factors regulating interaction of the von Willebrand molecule with binding sites on the platelet membrane. One may also anticipate the elucidation, within the next 5 years, of the properties of native human coagulant factor VIII and of the alterations of the molecule resulting from its exposure to thrombin. Major advances should also be made in understanding the structural abnormalities leading to impaired factor IX coagulant activity in variant factor IX molecules in hemophilia B.

New Activities. With the anticipated purification of the von Willebrand molecule subunit and the coagulant factor VIII molecule, efforts will undoubtedly increase over the next 5 years to isolate and characterize the genes for both of these molecules. After this accomplishment, exploration of genome analysis techniques in the diagnosis of hemophilia A and the carrier state in utero will be a logical step and should receive high priority.

It is difficult to predict whether cloning of the gene for hemophilia A will be accomplished within the next 5 years, and it is premature to speculate whether this development will lead to serious efforts to prepare coagulant factor VIII for therapeutic use by bacterial synthesis. Studies of physiological regulation of synthesis of the von Willebrand and coagulant factor VIII molecules and of mechanisms regulating their catabolism have lagged behind biochemical studies. They deserve increased emphasis in the next 5 years, as does the effort to determine the site of cellular synthesis of coagulant factor VIII.

A major effort is needed to discover the immunologic reasons why certain hemophilic patients develop antibodies to coagulant factor VIII whereas the majority of patients do not. This most pressing question needs to be answered for improving the care of patients with hemophilia A. Minimal, if any, basic investigations currently address this question. Means to bring persons together who are experts in the molecular biology of immune tolerance and in hemostasis should be designed. Fundamental work in this critical area deserves very high priority.

Current prothrombin complex concentrates still possess thrombogenic potential. Concentrates free of thrombogenic potential need to be developed. Application of monoclonal antibody techniques to preparation of a factor IX concentrate deserves initial evaluation.

Acquired Bleeding Disorders

Work currently in progress on mechanisms for induction of cellular procoagulant activities in monocytes and macrophages will improve understanding of the pathogenesis of intravascular coagulation in several disease states. Such efforts deserve continued high priority support. Measurement of the newly discovered vitamin K-dependent proteins C and S should elucidate their alterations in different disease states. The development of a prothrombin complex concentrate that would be safe to give to patients with chronic liver disease would significantly improve the management of gastrointestinal bleeding, which is a major cause of death, in such patients.

Thromboembolism

Thrombosis is the formation of an aggregate of platelets, fibrin, and entrapped cellular elements within the lumen of a blood vessel. A thrombus blocks or partially blocks the lumen of a blood vessel, with resultant impairment of flow of blood to or from an organ or tissue. An embolus is material that breaks from a thrombus and occludes a vessel downstream. The reactions involved in the formation of a thrombus are very similar to those of normal hemostasis. Essential to stanch bleeding from a severed vessel, they become harmful when they occur inappropriately within the lumen of a blood vessel.

Thrombosis can occur in arteries, in veins, and in the small blood vessels of the microcirculation. Arterial thrombosis almost always occurs locally at the site of an abnormality of the arterial wall, usually at a site where there has been a proliferation of smooth muscle cells, accumulation of fat, and disruption of normal wall tissues, such as an advanced atherosclerotic lesion. An acute arterial thrombosis in an atherosclerotic coronary artery is the usual cause for a heart attack; an arterial thrombosis within an atherosclerotic cerebral vessel is the usual cause for a stroke.

Venous thrombosis can develop secondary to vein wall damage or develop in a normal vein as a result of the combination of increased blood coagulability and impaired blood flow. Clinically significant venous thrombosis occurs primarily in the deep veins of the leg, often after surgery. Activated clotting factors circulate at the time of surgery and cause a thrombus to form in a venous arcade of the calf of an immobilized surgical patient. Once started, the thrombus can continue to grow, finally extending out of the calf veins into the main veins of the leg. Here the thrombus can interfere substantially with the flow of blood out of the leg and produce pain and swelling. More importantly, pieces

of the thrombus can break off and be carried through the right side of the heart to lodge in blood vessels in the lungs. Such pulmonary emboli cause a patient to develop acute respiratory distress and, all too frequently, sudden death.

In a variety of conditions including severe bacterial infections, complications of childbirth, and certain types of cancer, enough procoagulant material enters the circulation to cause fibrin to form in the circulating blood. This circumstance is known as disseminated intravascular coagulation (DIC). Fibrin can then be deposited throughout the capillaries of various organs, where usually it is cleared away rapidly, with reopening of the microcirculation, by the process of fibrinolysis. Occasionally, however, and particularly in the kidney, the capillaries remain blocked by fibrin, and infarction (death of tissue due to insufficient blood supply) follows. Some clinical syndromes in which one finds sudden renal failure, hemolytic anemia, and thrombocytopenia appear to result from this series of events.

Focal areas of small blood vessels can become inflamed. This condition, which is called vasculitis, is usually secondary to an immunologic event such as the deposition of immune complexes within the wall of the blood vessel. Thrombi may then form locally at the sites of the focal areas with resultant occlusion of vessels and ischemic tissue damage. This development is a second pathogenetic mechanism for organ damage secondary to thrombotic occlusion within the microcirculation of organs.

Thrombosis is not a disease in itself but a pathologic event that complicates the morbidity and contributes to the mortality of many clinical disorders. Almost without exception, individuals in Western societies, as they grow older, develop atherosclerosis of the arteries, which narrows the vessel lumen, reduces blood flow, and impairs the function of the organs or tissues they supply. This process occurs so regularly that it is viewed as a normal part of aging. Advanced atherosclerosis in an artery also sets the stage for an unpredictable, catastrophic event: the sudden formation of an acute, occluding thrombus at the site of atherosclerotic narrowing of an artery, with resultant infarction of the tissue dependent upon that artery for its blood supply.

Venous thromboembolism, which is ubiquitous, can be a lethal complication of a broad spectrum of medical and surgical diseases. Until recently, its hazard to a productive life has been masked by a lack of awareness of its true incidence and the scope of the disease states that it may complicate. Recent data, however, have documented its prevalence in our society. Venous thromboembolism is increasingly recognized as a complication of traumatic postoperative and postpartum states, acute myocardial infarction, acute pulmonary insufficiency, congestive heart failure, shock,

estrogen therapy, gram-negative sepsis, polycythemia vera, certain dysproteinemias, burns, and malignant tumors.

Thromboembolism induced by contact of the blood with foreign surfaces remains the major unsolved problem in the development of artificial organs or prostheses for extracorporeal circulation of the blood or for implantation within the body. Thus, a patient whose damaged heart valve is replaced by an artificial, plastic heart valve usually must receive lifelong anticoagulant therapy, with its attendant risks of bleeding, to prevent the even greater risk of a thrombus forming on the artificial valve. Progress in the development of a mechanical heart will remain limited until a truly nonthrombogenic surface is developed.

State of Knowledge in 1972

Almost nothing was known in 1972 of the mechanisms by which vascular endothelium maintains its remarkable nonthrombogenic property, and by today's standards, knowledge of the mechanisms of platelet activation was very limited.

It was generally recognized that loss of endothelial tissue, with exposure of blood to subendothelial tissue, is a common vascular response to a variety of injurious stimuli, and that platelets then adhere rapidly to the exposed subendothelium. Collagen in the subendothelium had been identified as one of the materials to which platelets adhere. The importance of the von Willebrand protein for adhesion of platelets to collagen was not yet appreciated.

Generation of fibrin was known to accompany the adhesion and aggregation of platelets at the site of vessel wall injury. It was appreciated that in flowing blood in an artery, this process leads to the formation of a thrombus consisting primarily of a white, platelet-fibrin "head" with no "tail." In stagnant blood in a vein, a thrombus is formed that has a small number of platelets in the head, and the thrombus is surrounded by a large red mass containing large numbers of entrapped red blood cells and resembling a stasis blood clot.

Platelet-fibrin thrombi adherent to a site of vessel wall injury were known to become incorporated into the wall of the vessel as healing occurs. Some investigators believed that this process represented an important pathogenetic mechanism for the development of atherosclerosis. Proliferation of smooth muscle cells and their migration into the intima had been identified as a major cause for the thickening of the vessel wall that occurs in atherosclerosis. The function of a mitogenic factor from platelets in initiating this process was yet to be discovered.

It was also known from animal experiments that immediately after the intravenous injection of several activated clotting factors, clamping a segment of the vein may result in a thrombus being formed in a vein that is distant from the injection site in which stasis was produced. It was inferred from these experiments that hypercoagulability, defined as entrance into the circulation of material capable of initiating the blood coagulation reactions, combined with stasis, is an important pathogenetic mechanism for venous thrombosis in human disease states.

Blocking the blood coagulation reactions by giving heparin was accepted as effective therapy for ongoing deep venous thrombotic diseases. Depressing the levels of the native vitamin K-dependent clotting factors--prothrombin, factor VII, factor IX, and factor X--by giving warfarin had also been established as effective therapy for deep venous thrombotic disease with pulmonary emboli. Similarly, it had been found effective in preventing venous thrombosis and pulmonary emboli after trauma and after orthopedic surgery. Whether the converse circumstance--that is, elevation of the concentration of native clotting factors--increased the risk for venous thrombosis was not known. A single family had been described in which elevated levels of factor V was associated with episodes of thrombosis in several family members. A second family had been described with an increased concentration of factor VIII coagulant activity and a similar tendency to spontaneous thrombosis.

Despite the efficacy of warfarin therapy, isolated factor VII deficiency, which presumably impairs extrinsic clotting but leaves intrinsic clotting intact, was known not to protect against venous thrombotic disease. Several families had been discovered in whom members with hereditary factor VII deficiency had experienced venous thrombotic events. Moreover, hereditary factor XII deficiency, which impairs blood coagulation initiated by the contact activation reactions but does not impair coagulation initiated by tissue factor, was also known not to protect against venous thrombotic disease.

Family studies had provided clues that defective fibrinolysis increases the risk of venous thrombotic disease. Thus, several families had been discovered in which hereditary dysfibrinogenemias were associated with an increased tendency to thrombosis. A single family had also been reported in which impaired release of vascular plasminogen activator was associated with repeated thrombotic episodes in several family members.

It had been known for several years that hereditary deficiency of antithrombin III, which results in plasma concentrations of antithrombin III in the 40 to 50 percent of normal range, substantially increases the risk for venous thrombosis. Antithrombin III had recently been shown to be identical to the

factor X_a inhibitor of plasma and had also been recognized as the plasma heparin cofactor. Substantial epidemiologic data had established that estrogens increase the risk of both venous and arterial thrombosis and were responsible for the increased incidence of thrombosis associated with the use of oral contraceptive agents. It was suggested that the increased thrombotic risk of estrogens stems from their ability to impair antithrombin III function.

In contrast to a general acceptance of the importance of altered blood coagulability in the pathogenesis of venous thrombosis, many investigators believed that altered blood coagulability is unimportant in the pathogenesis of arterial thrombosis. Little direct evidence was available for this point. Its primary support was derived from the disappointing results of large-scale anticoagulant therapy trials in which anticoagulant therapy had been found to have minimal, if any, effect in preventing myocardial infarction in patients with angina and in patients who had experienced a previous myocardial infarction.

An animal model for studying the pathogenesis of arterial thrombosis had just been developed in which a balloon catheter was introduced into the aorta and used to remove arterial endothelial cells selectively in vivo. Studies of factors regulating the subsequent deposition of platelets upon the deendothelialized aortic surface were just beginning. A model system for thrombosis was also in use with pigs in which the rate of platelet accumulation was measured in extracorporeal shunts. Studies of platelet and fibrinogen turnover had been reported as useful in evaluating thrombotic disorders in patients.

Interest was strong in the possible functions of altered platelet reactivity in the pathogenesis and therapy of arterial thrombosis. A number of pharmacologic agents had been identified that inhibited platelet function, such as aspirin, dipyridamole, PGE_1, and dextran. Little was known, however, about their mechanisms of inhibition. Despite this lack of knowledge, clinical studies had been undertaken or projected to evaluate antiplatelet drug therapy in transient cerebral ischemic attacks and in incomplete stroke, in the prevention of myocardial infarction, and in the prevention of thrombosis in artificial heart valves and in hemodialytic shunts. When added to warfarin therapy, dipyridamole had been reported to reduce the incidence of postoperative thromboembolism in patients undergoing cardiac valve replacement. Aspirin had been reported to protect against induced thrombosis in experimental dogs. Thus, one might expect that atherosclerosis would be less extensive or even nonexistent in a disorder in which platelet adhesion to subendothelium is diminished. The literature, however, contains a report of a postmortem study of 43 patients with von Willebrand's disease--a disorder in which platelet adhesion to subendothelium is diminished--where diffuse

atherosclerosis was found. Two of the patients had extensive coronary atherosclerosis and had died of myocardial infarction.

Streptokinase and urokinase were being used in the investigational treatment of venous thrombosis and pulmonary embolism. Fibrinolytic therapy had been reported to reduce the size of pulmonary emboli. There were scattered reports of the investigational use of fibrinolytic therapy in arterial thrombotic disorders.

Disseminated intravascular coagulation was recognized as a complication of a wide variety of clinical disorders, of which one is bleeding caused by consumption coagulopathy and by the antihemostatic effects of fibrin degradation products. Ischemic tissue damage, particularly of the kidney, was also recognized as an uncommon but serious clinical manifestation of disseminated intravascular coagulation that was found mainly in pediatric disorders and in complications of pregnancy. Microangiopathic hemolytic anemia was also known to be a rare complication of disseminated intravascular coagulation. Whether disseminated intravascular coagulation contributed significantly to the irreversibility of endotoxin shock in patients with overwhelming gram-negative septicemia was still being debated. Many clinicians were unsure of the importance of heparin, of replacement of coagulation factors and platelets, and of the combination of heparin and antifibrinolytic therapy in the treatment of disseminated intravascular coagulation in the different clinical circumstances in which it was encountered.

Interest was high in the development and use of newer diagnostic techniques for recognition of thrombosis and of disseminated intravascular coagulation. Although angiographic methods were being used increasingly for the diagnosis of arterial occlusive disease, noninvasive methods for detecting arterial occlusion were essentially nonexistent. Ventilation-perfusion scans had been introduced for the diagnosis of pulmonary emboli. The technique of ^{125}I-fibrinogen scanning had been devised to identify venous thrombi in the legs, and the use of this technique had disclosed a strikingly high incidence of postoperative thrombi in small calf veins in patients over 40 years of age. Trials of prophylactic low-dosage subcutaneous heparin administration were under way to determine if such prophylaxis significantly reduces the incidence of these thrombi.

Paracoagulation tests that are sensitive to fibrin monomers and early fibrin degradation products in plasma had been standardized for clinical use. Their usefulness and limitations as screening tests for the diagnosis of disseminated intravascular coagulation had been delineated. Their potential usefulness as a blood test for recognizing ongoing venous thrombosis was considered questionable but in need of further evaluation. Immunologic

tests for measurement of fibrin(ogen) degradation products were also being evaluated for their usefulness in the diagnosis and monitoring of intravascular coagulation and of venous thrombosis. Measurement of macromolecular fibrinogen complexes by gel-sieving techniques had been proposed as a test method for evaluating hypercoagulability, intravascular coagulation, and thrombosis.

Although heparin and warfarin were accepted as standard therapies for venous thrombotic disease and for mural thrombi in the heart and as probably effective therapies for transient ischemic attacks, the techniques of dosage administration and duration of therapy were far from standardized. A report had just appeared that continuous intravenous administration of heparin at a level that keeps the partial thromboplastin time at 1.5 to 2.0 times the normal value effectively controls ongoing thrombotic disease. The ways in which different prothrombin time-test methods and different types of thromboplastins can influence the delineation of a "therapeutic range" for warfarin therapy were not widely understood. How long anticoagulants should be used to treat an acute episode of venous thrombotic disease was not known.

Program Goals Through 1982

The broad goals as stated in the 1972 National Program and in the Fifth Report of the Director, 1978, were:

- Understand the interplay of the blood coagulation system, the platelets, the vessel lining, and the properties of flowing blood, as well as the role this interplay has in the development of thrombosis and atherosclerosis.

- Improve the diagnosis of, and therapy for, arterial thrombosis and the various clinical sequelae of this disease process in order to bring about its ultimate prevention.

- Enhance the basic knowledge of venous thrombosis in order to provide improved prophylactic therapy and patient care.

More specific long-term objectives of the DBDR also evolved over the past decade, partly from recommendations of the Blood Diseases and Resources Advisory Committee. Several of these objectives, which are related to blood vessel wall biology, the initiation and regulation of blood coagulation, and the activation of platelets, can be found in the dicussion of normal hemostasis. Other long-term objectives were:

- Devise specific methods to predict the development of deep venous thrombosis in groups of high-risk patients.

- Improve diagnostic accuracy for deep venous thrombosis using noninvasive methods, including thrombus-localizing agents, blood assays, and immunologic techniques.

- Encourage further research on anticoagulant agents, primarily heparin and vitamin K-antagonists, and their mechanism of action.

- Expand the knowledge of the fibrinolytic system, develop new fibrinolytic agents, and improve the therapeutic effectiveness of existing drugs.

Accomplishments Through 1982

The previously discussed spectacular advances in understanding normal hemostasis--the properties and function of endothelium, the activation of platelets, and the blood coagulation reactions and fibrinolytic reactions--also represent advances in understanding the pathogenesis of thrombosis. The accomplishments that are particularly pertinent to thrombosis include:

Synthesis of Prostacyclin. The discovery that endothelial cells synthesize a prostaglandin that inhibits platelet aggregation represents a key advance in understanding how vascular endothelium maintains its nonthrombogenic properties.

Altered Reactivity of Bound Thrombin. The discovery that the binding of thrombin to a material isolated from endothelium, which is called thrombomodulin, alters the substrate specificity of thrombin and causes it to activate protein C and to fail to clot fibrinogen or activate factor V represents a fundamental advance in understanding the mechanisms preventing the generation of fibrin on endothelium. It brings into focus the need to examine the effect of the surface of the endothelial cell upon the entire sequence of the blood coagulation reactions and upon the activity of the protease inhibitors that regulate blood coagulation.

Antithrombin III. Major progress has been made in understanding the importance of antithrombin III in the prevention of thrombosis. The primary structure of antithrombin III has been determined, its binding sites for heparin have been identified, and, at a detailed molecular level, the interaction of antithrombin III with the different serine protease coagulation enzymes has been analyzed in the presence and in the absence of heparin.

Protein C in the Regulation of Blood Coagulation. The discovery of protein C, its activation by thrombin bound to thrombomodulin, and the ability of its activated form, protein C_a, to inactivate factor V_a and factor $VIII_a$ represents a fundamental

advance in understanding the physiological regulation of blood coagulation. Of particular significance is the recent evidence that moderately reduced plasma protein C concentration is associated with an increased risk for thrombosis.

Platelet Mitogenic Factor. The identification of a low molecular weight protein in the platelet alpha-granules, which is released when platelets are activated, and which stimulates the growth of smooth muscle cells in culture, represents an important discovery linking platelets to proliferation of smooth muscle cells in vessel walls in the pathogenesis of atherosclerosis.

Animal Models. The development and use of new animal models represent a further substantial accomplishment of the past decade. Platelet consumption in a primate model has been used to study mechanisms of endothelial cell injury and the protection afforded by antiplatelet drugs upon such injury in the pathogenesis of experimental arteriosclerosis. In the rabbit, experimental thrombocytopenia, induced by platelet antibodies, has been shown to protect against the development of thromboatherosclerosis after mechanical injury to aortic endothelium. Pigs with severe von Willebrand's disease have been used as a model to study the effect of impaired adhesion of platelets to subendothelial tissue upon the degree of arteriosclerosis that develops after vascular injury and a high-fat diet. Immunofluorescent techniques utilizing antibodies to von Willebrand protein and to PF4 have been developed to study reactions of platelets and materials released from platelets with components of the vessel wall in the response of the blood vessel wall to injury.

Action of Oral Coagulants and Heparin. The understanding of the action of oral coagulants and heparin has advanced remarkably. It is now known that oral anticoagulants act as competitive inhibitors of vitamin K in the posttranslational gamma-carboxylation of glutamic acid residues on the vitamin K-dependent coagulation proteins; that oral anticoagulants affect the synthesis of two previously unknown proteins, protein C and protein S; and that only a small number of the molecules in preparations of heparin possess anticoagulant activity. Chemical analyses of the active material have revealed a unique sequence of monosaccharides clustered about a nonsulfated iduronic acid moiety, which appears to constitute the binding site of heparin for antithrombin III. The relatively inactive mucopolysaccharide fraction of heparin preparations has been found to suppress smooth muscle proliferation in animal models.

New Techniques. Another area of significant accomplishment has been the development of techniques for evaluation of the activation of platelets, the generation of fibrin, and the initiation of fibrinolysis in human thrombotic disease states. Of

particular importance has been the development of radioimmunoassays for fibrinopeptide A and for a polypeptide released by plasmin from the B chain of fibrinogen. Radioimmunoassays for PF4 and for beta-thromboglobulin have been devised and used to evaluate platelet activation in a variety of thrombotic disorders. Immunologic assays for prothrombin fragments, for complexes of antithrombin and thrombin, and for complexes of antiplasmin and plasmin have also been developed. Platelet turnover and fibrinogen turnover have been evaluated as indicators of thrombotic activity in a variety of clinical circumstances, such as in patients with artificial heart valves who are receiving different prophylactic antithrombotic regimens. These techniques would appear to have substantial potential for increasing the understanding of the pathogenesis of vascular injury, and of thrombosis in a variety of clinical disorders, and also for monitoring the effectiveness of antithrombotic prophylaxis and therapy.

Noninvasive Diagnosis. Substantial progress has been made in the evaluation of noninvasive diagnostic methods for venous and arterial occlusive disease. In the diagnosis of deep venous thrombosis in the legs, the combination of ^{125}I-fibrinogen scanning, for identifying thrombi in the calf and popliteal veins, and of Doppler sound-scanning or impedence plethysmography, for identifying thrombi in the deep veins of the thigh, has been found to approach the diagnostic accuracy of the invasive procedure of venography. Occular plethysmographic and ultrasound techniques have proven valuable for the screening evaluation of the patency of the carotid arteries; and platelet imaging, using ^{111}In-labeled platelets, has also shown promise for the noninvasive diagnosis of arterial thrombotic disease.

Antiplatelet Drugs. Certain drugs have been studied extensively both in laboratory experiments, to elucidate their mechanisms of action, and in large-scale clinical trials, to evaluate their possible usefulness as antithrombotic agents. Aspirin, for instance, was found to inhibit the oxidation of arachidonic acid by cylooxygenase, with resultant impairment of thromboxane A_2 synthesis in the platelet but also impairment of prostacyclin synthesis in the endothelial cell. Of importance for the design of antithrombotic therapy was the discovery that very small doses of aspirin may block thromboxane A_2 synthesis without significantly affecting prostacyclin synthesis. Unfortunately, extensive clinical trials were undertaken with large doses of aspirin before this information became available.

Evaluation of Antiplatelet Drugs. Three antiplatelet drugs have received extensive clinical evaluation: aspirin, dipyridamole, and sulfinpyrazone. In the dosage that was used, these agents--all of which could potentially inhibit the formation of platelet microemboli--had, at best, equivocal effects upon the morbidity and mortality of occlusive coronary artery disease. In

one large trial, aspirin was found to show some benefit in males only in reducing the incidence of complications of occlusive cerebral vascular disease. Although disappointing, these clinical trials have been important in putting into clinical perspective the limits of usefulness of platelet inhibitors in patients with manifestations of advanced arterial atherosclerotic disease.

Use of Heparin. Understanding of the clinical use of heparin has increased significantly. In an extensive study of post-operative patients over the age of 40 years, low-dosage prophylactic heparin in a schedule of 5,000 IU subcutaneously every 8 hours was found to reduce not only the incidence of positive ^{125}I-fibrinogen scans but also the incidence of fatal pulmonary emboli. Low-dosage heparin did not provide effective prophylaxis, however, for patients undergoing reconstructive hip surgery or open prostatectomy. Techniques for therapeutic administration of heparin to patients with established venous thrombosis have been increasingly standardized, and continuous intravenous administration, with monitoring by the partial thromboplastin time, has become widely used. Heparin-induced thrombocytopenia, with the accompanying threat of paradoxical thromboembolism, has been recognized as a side effect of heparin therapy. Its incidence, mechanism, and manifestations are under current study by a cooperative group from three Specialized Centers of Research in thrombosis organized by the DBDR.

Use of Oral Anticoagulants. New guidelines have been promulgated for the use of oral anticoagulants. With the commercial thromboplastins in use in the United States, a prothrombin time ratio between 1.5 and 2.0 has been increasingly accepted as the proper therapeutic range for the vast majority of patients. Techniques have been developed to control the quality of prothrombin time-test methods, and standard-reference thromboplastin preparations and standard-reference plasmas are available for this purpose. Clinicians have become increasingly aware of the importance of drug interactions in modifying the response to warfarin. A number of newer, widely used drugs have been found to modify warfarin dosage, including cimetidine, trimethoprim-sulfa combinations, and carbamazepine.

State of Knowledge in 1982

The accomplishments of the past 10 years have yielded a new fundamental understanding of relations between thrombosis and atherosclerosis; of mechanisms by which vascular endothelial cells maintain their nonthrombogenic properties; of mechanisms of platelet activation and response that are important for the initiation of thrombosis; of the function of blood coagulation and its regulation in the pathogenesis of thrombosis; and of mechanisms

for initiating and limiting fibrinolysis in the modulation of thrombosis. Although basic discoveries have remarkably advanced the understanding in each of these areas, they have also increased the awareness of scientists of how very much more remains to be learned.

Increasing evidence supports the importance of platelets in the pathogenesis of atherosclerosis. A sequence for the development of an atherosclerotic lesion has been formulated in which:

1. Vessel wall injury results in focal loss of endothelial cells.

2. Platelets adhere to exposed subendothelial connective tissue and then to each other to form aggregates.

3. Platelets release their granule constituents, which then interact with elements in the blood vessel wall.

4. Platelet products stimulate migration of smooth muscle cells into the intima and their proliferation.

5. Connective tissue matrix is increased through synthesis of collagen, elastic fiber proteins, and proteoglycans.

6. Lipid accumulates both intracellularly and extracellularly.

Although good evidence exists that in experimental animals thrombocytopenia inhibits development of acute atherosclerotic lesions after vascular injury, evidence is less clear that inhibition of platelet function also inhibits the development of atherosclerotic lesions. Severe von Willebrand's disease reportedly reduces the severity of atherosclerosis that has been experimentally induced in pigs; mild von Willebrand's disease does not protect against atherosclerosis in humans. Information on the prevalence and severity of atherosclerosis in humans with severe von Willebrand's disease is not available.

The interaction of platelets and platelet components with the vascular wall changes with time after injury. Platelets initially adhere in large numbers to exposed subendothelium, and after a period of time, fewer and fewer platelets adhere. The reason is unknown but perhaps related to a decreased availability of von Willebrand protein at the vessel wall. Similarly, smooth muscle cells initially respond to released platelet mitogenic factor by migrating and proliferating. With time, proliferation ceases despite the lack, as yet, of a new endothelial cover. The cause for the cessation of proliferation is also unknown. Studies of the reactions that govern the changing reactivity of the blood

vessel wall after injury seem feasible with presently available techniques, and are of fundamental importance.

A number of factors have now been identified that may contribute to the nonthrombogenic properties of endothelium: synthesis of prostacyclin, binding of thrombin to endothelium with resultant alteration of its substrate specificity and rate of inactivation; potentiation of the activity of antithrombin III by its interaction with endothelial cell surface heparan sulfate; release of vascular plasminogen activator; and inhibition of ADP by an endothelial surface ADPase. The knowledge and methods are available to design further studies to evaluate the relative importance of each of these factors under different physiological and pathological circumstances. Of particular importance will be the determination of whether selective inhibition of prostacyclin synthesis can, of itself, lead to thrombosis, since this possibility has been postulated as the cause of thrombosis in several human disease states.

Awareness is increasing of the influence of rheologic factors upon the structure and localization of thrombi. In normal vessels, blood has a characteristic tendency to flow in concentric cylindrical layers with the innermost stream moving most rapidly and each successive layer moving more slowly, with minimal flow at the vessel wall. Since blood becomes more viscous at low rates of flow, a finite level of force ("yield stress") is required before it starts to flow. This force increases as either hematocrit or fibrinogen concentration increases. Such increases are thought to be one mechanism for the increased thrombotic tendency found in clinical conditions in which blood viscosity is increased.

Irregularities in arterial walls produce a turbulent pattern of flow that promotes formation of thrombus. Platelets collide in such areas, especially if a vortex forms. Because velocity in the vortex close to the vessel wall is minimal, formed elements have little tendency toward inward radial migration. Thus the likelihood is increased that a platelet mass, once formed, will adhere to the wall. Further knowledge of the influence of such rheologic factors upon the pathogenesis of both atherosclerosis and thrombosis is needed, and the DBDR is currently attempting to foster interest in biophysicists and engineers in this area of research.

The mechanisms for activation of blood coagulation in different disorders associated with disseminated intravascular coagulation have been clarified. Gram-negative endotoxin has been shown to cause monocytes to acquire surface tissue-factor activity, and this activity is thought to induce blood coagulation in patients who have gram-negative endotoxemia. A material that directly activates factor X has been isolated from a malignant

tumor and characterized; it may be important for inducing coagulation in patients with metastatic malignancies. The prevalence and clinical effects of DIC in different clinical disorders have now been well delineated. Head trauma with destruction of brain tissue has been found to be associated with a high incidence of defibrination, which has potentially very serious consequences. The indications for the use of heparin in DIC have been narrowed to a few specific clinical circumstances. Endotoxin shock and DIC are now known to be independent, unrelated manifestations of endotoxemia, and heparin therapy has not proved beneficial in the treatment of endotoxin shock.

Hypercoagulability is generally recognized as important in the pathogenesis of venous thrombosis. The recent reports of myocardial infarction in hemophilic patients with factor VIII inhibitors who were treated with prothrombin complex concentrates may mean that under some circumstances hypercoagulability is also important for the pathogenesis of arterial thrombosis. An increasing number of family studies have clearly established that antithrombin III deficiency represents a major risk factor for venous thrombosis. The understanding of antithrombin III structure, function, and kinetics in vitro has advanced to the detailed molecular level. Studies to probe the mechanisms that regulate antithrombin III activity in vivo are beginning.

Very recent evidence suggests that protein C deficiency also represents a major risk factor for venous thrombosis. Knowledge of protein C structure, activation, and mechanisms of inhibition of factor V_a and factor $VIII_a$ is still limited but should increase rapidly. Activated protein C has also recently been shown to stimulate endothelium to release plasminogen activator. The significance of this observation for the pathogenesis of thrombotic disorders requires further study.

An anticoagulant that appears to interfere with the reaction between synthetic phospholipids and clotting factors in in vitro clotting systems is found in some patients with lupus erythematosus and in many other types of patients as well. This so-called "lupus anticoagulant" does not cause clinical bleeding. Paradoxically, there is an increased incidence of thrombosis in lupus erythematosus. The mechanism of this phenomenon should be studied for its value in the prevention of thrombosis.

A number of test methods have been developed to measure plasma materials that would reflect in vivo activation of platelets, of blood coagulation, or of fibrinolysis in patients. The methods include measurement of circulating platelet aggregates and immunologic assays for PF4, beta-thromboglobulin, and fibrinopeptide A. Techniques have been perfected to prevent platelet release or nascent clotting during preparation of the plasma.

Such problems plagued early studies. Therefore, with attention to technique, assays can now be reliably interpreted as reflecting in vivo conditions. A number of descriptive studies have been undertaken in which circulating platelet aggregates, PF4, beta-thromboglobulin, or combinations of these tests have been performed on groups of patients with different types of thrombotic or prethrombotic disorders. At times, positive results have been interpreted as indicating a causal role for platelet activation in the pathogenesis of a disorder. Positive results, however, could equally well reflect an effect of the disorder. At present, this is an area of considerable investigative activity, but the importance of such indicators of platelet activation in diagnosis or in monitoring therapy is not yet clear.

Fibrinopeptide A assays and assays for the fibrin degradation products, such as fragment E, have been evaluated for their usefulness as screening tests for active venous thrombosis. These tests are of considerable potential interest in that a negative test in a symptomatic patient may obviate the need for further extensive or invasive evaluation. Methods, however, are presently difficult and time consuming, and simpler techniques are needed if these assays are to become generally useful. If simple methods can be developed, fibrinopeptide A assays would seem ideal for evaluating the effectiveness of anticoagulant therapy in halting ongoing thrombosis.

Advances in knowledge of the structure of the active material in heparin preparations and of the different activities of low and high molecular weight heparins provide a scientific basis for the development of better heparin preparations. Knowledge of the structure also provides a basis for the development of methods for the chemical synthesis of heparin. Small peptides with powerful in vitro anticoagulant activity have been developed for laboratory use in studies of prothrombin activation and of fibrin polymerization. Such materials need in vivo evaluation in animals to determine their potential use as clinical anticoagulants.

Understanding has grown of the importance of heparin and warfarin in the management of venous thrombosis. Evidence has been obtained to document the need to continue anticoagulant therapy for a number of weeks after an acute proximal venous thrombotic event to prevent recurrence. A technique for determining a schedule of dosage of subcutaneous heparin that would be effective in replacing warfarin for this period of time has recently been developed.

Interest has grown in the use of fibrinolytic therapy in clinical thrombotic disease, but recent reports of pulmonary emboli after use of streptokinase in patients with deep venous thrombosis have raised concern. Of particular interest has been

the synthesis in tissue culture of sufficient vascular plasminogen activator for its evaluation as a fibrinolytic agent in experimental animals. This material has the major potential advantage of selectively activating plasminogen associated with fibrin. Thus secondary fibrinolysis can be permitted without the complications of bleeding of simultaneous primary fibrinogenolysis. Application of streptokinase, by means of a catheter, directly to the site of an arterial thrombus is being evaluated in a number of clinical studies, particularly for the possible salvage of myocardial function in patients with the very recent onset of coronary thrombosis.

Program Goals 1982 to 1987

- Enhance basic knowledge of the biology of the vascular wall in relation to the pathogenesis of thrombosis and atherosclerosis, including correlation of data from tissue culture in animal model systems.

- Assess the participation of thrombosis in the pathogenesis of atherosclerosis, including the effects of thrombocytopenia, of decreased availability of von Willebrand protein, of agents that inhibit platelet aggregation, and of materials that affect the blood coagulation and fibrinolytic reactions.

- Continue studies, as they relate to the pathogenesis of thrombosis, of the interaction of endothelial cell surface membrane materials with platelet constituents and with plasma coagulation and fibrinolytic proteins.

- Expand studies, at the molecular level, of the mechanisms by which the known inhibitors of blood coagulation, antithrombin III, alpha-2-macroglobulin, and protein C_a, protect against thrombosis, and search for and characterize added inhibitors of hemostasis important for such protection.

- Enhance basic knowledge of initiation and control mechanisms of the fibrinolytic process as they relate to the pathogenesis of thrombosis, including the effects of protein C_a and other materials generated during hemostasis upon the release of vascular plasminogen activator.

- Characterize fibronectin and its relationship to processes involved in the pathogenesis of thrombosis and atherosclerosis.

- Encourage studies of rheological factors that influence thrombosis, including development of new models to evaluate rheologic effects upon platelet-vessel wall interactions, and studies of how alterations of endothelial cell surface materials may affect rheological parameters.

- Develop clinical test methods for the evaluation of deficient or reduced fibrinolysis as a risk factor for thrombotic disease.

- Continue evaluation of tests reflecting platelet activation, such as circulating platelet aggregates and release of PF4 and beta-thromboglobulin; tests reflecting activation of blood coagulation, such as fibrinopeptide assays and assays for prothrombin fragments; and tests reflecting activation of fibrinolysis, such as specific early and late fibrin degradation products, in the prevention, diagnosis, and management of clinical thrombotic disorders.

- Continue development and evaluation of noninvasive diagnostic methods for the diagnosis of both arterial and venous thrombosis.

- Continue investigations of the structure of heparin, the mechanisms of its antithrombotic action, and its effects upon platelets, and encourage the development of improved preparations of heparin for clinical use.

- Evaluate the possible clinical usefulness of prostaglandins in prevention or management of thrombosis, including protection against platelet activation and thrombosis during procedures involving exposure of blood to foreign surfaces such as open heart surgery and hemodialysis.

- Continue to develop and assess other agents or measures for prevention of thrombosis, including new platelet inhibitors and anticoagulant materials, new fibrinolytic activators, and new methods of administration of fibrinolytic agents.

- Continue clinical and epidemiologic studies to identify environmental factors and factors related to disease and age that influence or predict the risk of developing atherosclerosis, arterial thrombosis, and venous thrombosis.

Research Activities 1982 to 1987

Current Activities

The Division's current program on thromboembolic disorders includes basic investigations, which are concerned with the pathogenesis of thrombosis and its relation to atherosclerosis, and clinical investigations, which are concerned with the prophylaxis, diagnosis, and treatment of thrombotic disorders. As thrombosis represents an aberration of normal hemostasis, many of the basic investigations discussed under normal hemostasis are directly relevant to the pathogenesis of thrombosis.

Atherosclerosis is the usual vascular lesion underlying arterial thromboembolism. A number of laboratories are investigating the factors involved in atherogenesis, including the relation between atherosclerosis and thrombosis. The Division of Heart and Vascular Diseases, NHLBI, supports the majority of these laboratories. The thromboembolic disorders program of the DBDR supports a small number of studies directed to the relation between alterations in hemostatic factors and the pathogenesis of atherosclerosis. These include three projects in which the pig model of von Willebrand disease is being used to investigate the effect of impaired platelet adhesion to vascular tissues upon the pathogenesis of experimental atherosclerosis, a study concerned with the factors regulating endothelial cell regrowth after balloon injury to arteries and veins, three studies utilizing tissue cultures of vascular wall cells to study factors regulating prostacyclin synthesis and release and the effect of peptide growth factors upon smooth muscle replication, and at least three studies of the effect of environmental factors in inducing vascular injury and in altering endothelial cell function.

A prominent characteristic of the approximately 25 projects that the DBDR supports on the pathogenesis of thrombosis is the attempt to characterize the effect of different predisposing factors, including perturbation of blood flow, of coagulation factors, of platelet function, and of cellular function, particularly endothelial cell function, and the occurrence of protease inhibitors, other regulatory proteins, and fibrinolytic factors. A remarkably wide range is covered--from studies of factors stabilizing platelet membranes to factors regulating protein C activation. Several studies are concerned with the evaluation of alterations of hemostatic factors as pathogenetic mechanisms for thrombosis in different clinical disorders, such as leukocyte tissue factor in endotoxemia, von Willebrand protein and platelet activation in diabetes, and abnormalities of platelets and coagulation factors in the nephrotic syndrome.

Turnover of isotopically labeled platelets, immunoassays of PF4 and beta-thromboglobulin, and imaging techniques utilizing ^{111}In-labeled platelets are being utilized in approximately 10 laboratories in a variety of studies of platelet activation in vascular injury in animals or of platelet activation in human thrombotic disorders. Assays of fibrinogen or fibrin derivatives, such as fibrinopeptide A assays, are being used in animal and clinical studies. A total of 13 grants support work related to the development and evaluation of such techniques for clinical diagnosis. In some medical centers, PF4 and beta-thromboglobulin assays have already been introduced into general medical practice. Nevertheless, it would appear that the usefulness of such markers of platelet activation could benefit from further clinical evaluation.

The DBDR continues to support studies of the effect of aspirin and other inhibitors of arachidonic acid transformation upon platelet and endothelial cell function. New inhibitors of platelet aggregation are being developed for possible clinical evaluation, including stable analogs of prostacyclin. Prospective, controlled clinical trials of preventive measures, including aspirin, in surgical patients at risk of venous thrombosis are being supported.

Seventeen current projects are concerned with heparin, antithrombin III, or the interaction of heparin and antithrombin III. In some, biochemical and biophysical methods are being used to determine the structural features of heparin required for its anticoagulant activity and for its antiplatelet activity. One goal of this work is the development of a heparin fraction of reproducible activity and fewer untoward side effects. Pharmacokinetic and pharmacodynamic studies of heparin are in progress. The interaction of antithrombin III and heparin is being studied by a variety of techniques, both to characterize the nature of the interaction and to define the antithrombin-serine protease interaction that heparin most influences. Clinical studies of the effect of different heparin preparations upon the catabolism of antithrombin III are also in progress.

The number of projects concerned with the use and actions of warfarin has declined steadily over the past several years. Six projects are currently supported. They vary from studies of the drug itself to studies of the usefulness of immunologic assays of decarboxylated prothrombin as a technique for monitoring warfarin therapy. The Division also supports an experimental animal study of immobilized streptokinase as a thrombolytic agent and a study of patients in whom fibrinolysis has been induced with urokinase.

Nine projects are being supported that are directed to the development and evaluation of new drugs or plasma proteins for

possible therapeutic use in the thrombotic disorders. Materials being studied include inhibitors with specificity for one or more of the clotting or lytic proteases, snake venoms, and the stable analogs of prostacyclin.

Very few studies are being supported that are directed to the evaluation of present methods of management of venous thrombotic disease. A single retrospective study has been initiated in which hospital records are being reviewed to evaluate the influence of duration of oral anticoagulant therapy following pulmonary embolism or deep venous thrombosis upon the risk of recurrent thromboembolism following cessation of therapy.

New Activities

There are currently only a few rheologic studies of thrombosis. The Division has encouraged and plans to continue encouraging increased interest in this area of research.

The absence of prospective clinical studies of current management of venous thrombosis is disturbing. Important practical clinical questions remain unanswered. After beginning heparin, when should warfarin be started? How long should anticoagulant therapy be continued in the different clinical situations in which venous thrombosis is encountered? What is an adequate therapeutic range for the prothrombin time when warfarin is given for long-term prophylaxis against recurrence after an acute attack has subsided? How long should anticoagulant therapy be continued in patients who have had more than one recent attack of venous thrombotic disease? Should subcutaneous heparin replace warfarin for such long-term therapy? Have the indications and hazards of fibrinolytic therapy for acute venous thrombotic disease been critically delineated? The answers to such questions will require the availability of large numbers of patients and, therefore, a group study. The possibility of contract support for such a group study deserves careful consideration.

4. Red Blood Cells and Their Disorders

Contents

4. Red Blood Cells and Their Disorders

This report covers five areas of research supported by the Division of Blood Diseases and Resources of the National Heart, Lung, and Blood Institute: erythropoiesis and stem cell disorders, circulating red cells, molecular and cellular biology of hemoglobin, sickle cell disease, and the thalassemia syndromes. In preparing this report,* the working group took into consideration only the major advances of the last decade and those that had a direct impact on patient care. Because an in-depth review of the general area of red cell disorders (anemia and polycythemia) is outside the scope of this report, several important categories of anemia are not discussed, such as the nutritional deficiency anemias (iron, B_{12}, folic acid) and the anemias secondary to chronic diseases (renal failure, malignancy, endocrine disorders, and chronic inflammation).

It has become clear from the experiences of the past that in this field, developments having a major impact in medicine and biology came from discoveries that were not included in the projections or goals set by expert committees of the early 1970's. It is the consensus of the scientists and investigators who contributed to this report that the system of NIH-supported research should operate with the degree of flexibility that encourages the maximum utilization of the initiative, motivation, and creative imagination of scientists and clinical investigators. This committee does not think that the accomplishments of centrally directed research can match the achievements of the highly competitive NIH system of research grants.

The state of knowledge in the 1990's will greatly depend on whether a critical mass of new talented clinical investigators and scientists enter the field during the 1980's. That number will be determined by the opportunity for new investigators to pursue and

*The chairpersons of the Red Cell Working Group and staff of the DBDR gratefully acknowledge the opportunity to review a draft report of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases: Evaluation of Research Needs in Hematology 1981, prepared under the overall chairmanship of Ernst R. Jaffe. Of considerable help and relevance were chapters I [Hematopoiesis, Normal], II [Hematopoiesis, Abnormal], and VI [Circulating Erythrocytes].

achieve their goals through the existing mechanism of research grants.

Red Blood Cells

Structure and Function

The human red blood cell performs the crucial function of transporting oxygen from the lungs to the tissues of the body. Human red cells contain abundant quantities of hemoglobin, which is a pigmented protein that carries the oxygen. Hemoglobin is the major intracellular protein of the circulating red blood cell, and it accounts for over 95 percent of the total soluble protein of the mature erythrocyte. The hemoglobin molecule is a tetramer of four polypeptide chains: two alpha chains and two nonalpha chains, which in adult hemoglobin (Hb A) are beta chains, in minor adult hemoglobin (Hb A_2) are delta chains; and in fetal hemoglobin (Hb F) are gamma chains. Hb F is the major hemoglobin synthesized by the fetus. Although oxygen transport is its primary function, the human red cell is equipped with complex metabolic machinery (enzymes) to meet its energy requirements. A complex membrane or "envelope" encases the cell and maintains its shape and integrity.

The energy requirement of the red blood cell is modest, and it is met through the metabolism of glucose by way of the anaerobic glycolytic pathway. The glucose metabolized to lactate by this pathway (about 90 percent) results in the production of adenosine triphosphate (ATP), the major energy-rich compound, and 2,3-diphosphoglycerate (2,3-DPG), the intermediate that facilitates oxygen release from red cells to tissues. About 5 to 10 percent of the glucose is normally shunted through the aerobic hexosemonophosphate (HMP) pathway, which protects both hemoglobin and the membrane from oxidant injury.

The primary structure of the red blood cell membrane is a lipid bilayer consisting of phospholipids, cholesterol, and glycolipids. The lipid bilayer contains 10 to 12 major protein species that fall into two classes: integral and peripheral. Integral membrane proteins, which include membrane antigens (Rh blood group), receptors, and transport proteins, penetrate or traverse the lipid bilayer and interact with the hydrophobic lipid core. The major peripheral membrane proteins (spectrin, actin, ankyrin, and band 4.1) are arranged in an extensive fibrous network that laminates the inner (cytoplasmic) membrane surface and interacts with the integral proteins to form the membrane skeleton.

A defect of any of the major components of the red cell (hemoglobin, enzymes, and membrane) can lead to serious sequelae.

The major clinical disorders and diseases of red blood cells usually reflect a defect in one of these components.

Erythropoiesis is the process that maintains the production of adequate numbers of circulating red cells. It is the process by which the bone marrow produces and delivers erythrocytes and maintains their concentration in the blood within well-controlled limits. In adult humans, a major part of normal hematopoiesis occurs exclusively in the bone marrow. Normal hematopoiesis (including erythropoiesis) begins with the pluripotential myeloid stem cell and, after differentiation and maturation, culminates with the production and release of functional blood cells. Erythropoietin, which is the major physiologic regulator of erythropoiesis, is elaborated in response to alterations in oxygen delivery to the kidney. A number of clinically important red cell disorders result from disturbances in the maturation and production of erythroid stem cells and in the production of erythropoietin.

Red Blood Cell Disorders

Abnormal erythropoiesis can result in an overproduction in the number or function of hematopoietic stem cells (polycythemia vera), a deficiency in erythroid stem cells (pure red cell aplasia), or a deficiency in the number or function of several classes of hematopoietic stem cells (aplastic anemia). Aplastic anemia is a serious illness, and approximately 75 percent of patients with severe aplasia die of their disease. Aplastic anemia has assumed increased importance in medicine; it is one of the few hematologic diseases that in carefully selected cases can now be successfully treated and cured with bone marrow transplantation. The results achieved are being extended to malignant disorders (acute myelogenous leukemia [AML], acute lymphocytic leukemia [ALL]) and may be applicable to other nonmalignant hereditary hematologic diseases such as sickle cell anemia, thalassemia major, the hereditary enzymopathies (G-6-PD deficiency), and hereditary immunodeficiency disorders.

Approximately 20 inherited enzymatic defects of the erythrocyte have now been described. These defects result in metabolic derangements that shorten the life span of the cell and frequently cause anemia. The importance of the most common red cell enzymopathy, G-6-PD deficiency, is underscored by the fact that it is found in approximately 13 percent of black males and 20 percent of black females and is common in certain other racial and ethnic groups. In all, 200 million people in the world are affected by this genetic deficiency. Since individuals with it may develop severe anemia if exposed to certain drugs, including antimalarials, furadantin, and some sulfonamides, anemia can theoretically be prevented in such individuals by screening for the defect and avoiding the administration of drugs known to cause this problem.

The red cell membrane, the "envelope" which encases the human red cell, has been the focus of investigation during the past decade. Its importance in clinical medicine lies in the fact that changes in red cell membrane properties are the primary cause of many important hemolytic anemias. In addition, physiologic alterations occurring as a result of metabolic depletion and aging limit the duration of storage of banked blood for use in transfusions and determine the life span of the circulating red cell. These disturbances may be inherited, may be acquired after exposure to drugs, toxins, antibodies, and complement, or may develop as a result of nutritional deficiences. Investigation of membrane protein synthesis and the study of alterations in membrane structure and function are important in elucidating the changes that accompany the red cell aging process as well as a variety of pathologic states.

The study of hemoglobin and the various hemoglobinopathies has served as a prototype in the application of the tools of modern biochemistry, genetics, and cell biology to the understanding of human diseases. As of 1982, over 350 human hemoglobin variants have been described. During the past 30 years, the study of normal hemoglobin and its variants has provided new insights into the relationships between the structure and function of proteins, the pathophysiology of oxygen transport, and the mechanisms underlying red blood cell destruction. The study of the mutation that results in the production of sickle hemoglobin has made it possible to understand why patients afflicted with sickle cell anemia experience the many complications of this disease. The protean clinical manifestations of this disorder, including the recurrent vasooclusive phenomena (painful crises and chronic organ damage) as well as the severe hemolytic anemia itself, can be attributed to a specific molecular lesion: the subsitution of valine for glutamic acid at the sixth position of the beta chain of hemoglobin.

Sickle cell anemia (Hb SS disease), which is a worldwide health problem, is the most prevalent form of hereditary hemolytic anemia in the United States. It is estimated that approximately 1 in 500 black Americans (60,000) has the anemia and that 1 in 12 is a carrier or has sickle cell trait. As a result of the lifelong anemia and its disabling and debilitating systemic sequelae, sickle cell anemia imposes a major burden on the nation's economy and medical resources.

The other major hemoglobinopathy of worldwide importance is thalassemia. The thalassemia syndromes are hereditary hemoglobin disorders that result from diminished or absent synthesis of adult hemoglobin in red cells. An average of 5 to 7 percent of Americans of Mediterranean ancestry and approximately 1 percent of black Americans are carriers of the beta-thalassemia gene.

Individuals afflicted with homozygous beta-thalassemia (Cooley's anemia) have a catastrophic illness that requires repeated blood transfusions and repeated administration of expensive drugs to remove the excess iron that results from frequent transfusions. While the prevalence of Cooley's anemia in the United States has been estimated to involve fewer than 1,000 persons, the disease is a major drain on medical resources and the nation's blood supply. In several states, the annual total cost of routine care for a child with Cooley's anemia is approximately $8,000 per year.

Impact of Red Blood Cell Research on Other Fields

Because they are abundant and easy to obtain from human donors, red cells have proven to be an extremely useful tissue for investigation, and studies of red cells and their disorders have had a major impact on other fields of medicine. Red cells can be used in the study of diseases that are not primarily of a hematologic nature. Alterations in red cell membrane structure and function have been described in a variety of pathologic states such as muscular dystrophy, cystic fibrosis, hypertension, and obesity. The membrane of the red cell can be employed for the identification of receptors to hormones and drugs. Red cells can be used for the diagnosis of a number of genetic diseases such as galactosemia, certain of the immunodeficiency states, and for the demonstration of the effects of gene dosage in the trisomic conditions. In addition, the red cell has been used to monitor the clinical course of diabetes by the measurement of Hb A_{1c}.

The delineation of the biology of the hemoglobin system in thalassemia has provided insight for understanding human genetic disease in general. Research efforts in thalassemia have enhanced the understanding of the organization and expression of individual genes on chromosomes. This information is now being applied to other common disorders caused by abnormal gene structure or function, including sickle cell disease, diabetes, and cancer. The development of methodologies using recombinant DNA technology and the progress achieved as a result in genetic counseling and prenatal diagnosis in sickle cell disease may well serve as a model for other hereditary diseases, including hemophilia, inborn errors of metabolism, diabetes, and cancer.

Erythropoiesis and Stem Cell Disorders

Erythropoiesis, which is the process by which the bone marrow produces and delivers red blood cells and maintains their concentration in the blood within well-controlled limits, is a major part of normal hematopoiesis. In adult humans, it occurs exclusively in the marrow.

Hematopoiesis results from a series of events of differentiation and maturation that begin at the level of the pluripotential myeloid stem cell and result in the production and release of functional blood cells--red cells, several forms of white cells, and platelets. The understanding of normal and abnormal hematopoiesis advanced dramatically in the last 25 years as a result of the advent of assays for morphologically unrecognizable, but functionally defined, hematopoietic progenitor cells both in experimental animals and in humans. These progenitor cells have been referred to generically as colony-forming cells. Such cells may form colonies in the spleens of heavily irradiated rodents or in semisolid media under specified conditions and in the presence of selected growth factors. The techniques for growing and assaying these cells have permitted the development of methods to quantify various hematopoietic progenitor cells as well as to define their physical and biologic properties.

Abnormalities in the hematopoietic process may lead to the production of abnormally high or low numbers of one or more of the several types of blood cells produced in the bone marrow. Some diseases that result in reduced production of red blood cells, which is the primary concern of this discussion, include pure red cell aplasia, Diamond-Blackfan anemia, transient erythroblastopenia of childhood, refractory anemia, and acquired sideroblastic anemia. In aplastic anemia, the numbers of white cells and platelets, in addition to red blood cells, are also reduced. Aplastic anemia comprises a heterogeneous group of serious, life-threatening disorders that are usually idiopathic in nature but occasionally can be attributed to bone marrow damage resulting from exposure to drugs, chemicals, toxins, radiation, infection, or viral diseases such as hepatitis.

Two disorders of erythropoiesis that are characterized by the production of abnormal red blood cells are paroxysmal nocturnal hemoglobinuria and polycythemia rubra vera (PRV). In the former, the abnormal red cells are subject to episodic destruction in the bloodstream. In the latter, a harmful excess of red blood cells is produced, and it is frequently accompanied by the production of too many white cells and platelets. Both of these conditions have been shown to be clonal disorders of the hematopoietic stem cells.

Although these disorders are relatively infrequent, the severe and long-term illnesses they produce have a significant impact on health resources.

State of Knowledge in 1972

Normal Erythropoiesis

The regulation of hematopoiesis was understood only in general terms in 1972, and there were very limited opportunities for experimentation and clinical investigation. It was recognized that there are pluripotential hematopoietic stem cells that can be cloned in the spleens of irradiated mice. In that system, these cells produce distinct colonies of red blood cells, white blood cells, megakaryocytes, or mixtures of two or more of these types of cells. It was also well recognized that several types of white blood cells can be cultured in semisolid media, but there had been only limited success in culturing red cell progenitors in mice. No human red cell progenitors had been cultured. It was recognized that the hormone erythropoietin (EP) has an important function in the production of red cells and the control of their number, but the molecule had not been purified and could be assayed only by bioassays, which were cumbersome, expensive, and imprecise.

Abnormal Erythropoiesis

Investigations of hypoplastic and aplastic anemia in 1972 were mostly descriptive and empirical. There was little understanding of the pathogenesis of any of the forms of abnormal erythropoiesis mentioned above. Treatment of aplastic anemia consisted of supportive therapy in the form of transfusions for anemia and antibiotics for infections and of numerous other empiric therapies of little value.

Program Goals Through 1982

In the stated DBDR goals in 1972, no mention was made of hematopoietic stem cells. This absence is a reminder that most scientific discoveries result from astute, inquisitive investigators following up on observations that are frequently unrelated to the original purpose of the research project.

Goals as defined at various times from 1972 through 1982 included:

Normal Erythropoiesis

- Understand red cell proliferation at the molecular and cellular levels, especially through biochemical characterization and determination of the mode of action of

erythropoietin, and apply this knowledge to the prevention, diagnosis, and treatment of red blood cell diseases due to proliferative malfunction.

- Purify and distribute erythropoietin for research purposes.
- Develop better assay methods for erythropoietin, and particularly a reliable radioimmune assay.
- Elucidate the mechanisms for erythropoiesis and the regulatory factors governing erythropoietin production.
- Develop erythropoietin preparations suitable for use in controlling human diseases.
- Characterize the nature and function of stem cells through in vitro and in vivo studies.

Abnormal Erythropoiesis

- Develop knowledge of the underlying causes of aplastic and refractory anemias so as to permit improved treatment; develop information concerning the natural history of these diseases.

Accomplishments Through 1982: Normal Erythropoiesis

Cell Biology

Detection and Assays of Progenitor Cells. In the early 1970's, it was recognized from transplantation studies of irradiated mice that the bone marrow contains pluripotential hematopoietic stem cells. It was also recognized that the marrow contains several white blood cell progenitors that can be cultured in semisolid media. In 1972, a clonal assay for murine red-blood progenitor cells was first introduced. Since then, studies have demonstrated that several classes of erythroid progenitor cells from rodents and humans can be grown. Currently, at least three types of progenitor cells with erythroid commitment are recognized: erythroid colony forming units (CFU-E), which are most likely the immediate precursors of the erythroblast; erythroid burst forming units (BFU-E), which are considered to be more primitive cells than CFU-E's and give rise to CFU-E's; and the primitive progenitors bipotential or tripotential BFU's, which have the potential of differentiating into and providing the progenitors of white cells and megakaryocytes in addition to red

cell progenitors. These classes of progenitor cells have been inferred from the kinetics of cellular proliferation and from the cellular composition of colonies formed in clonal cultures. Many characteristics of these progenitor cells, such as their requirements for erythropoietin and other growth factors, have been defined, but the cells have not yet been isolated or identified.

Stem Cell Cultures. Until 1972, investigation of pluripotential hematopoietic stem cells could be made in vivo only by manipulating hematopoiesis in intact mice and by studying colonies of blood cells that grew in the spleens of irradiated mice transplanted with bone marrow cells. During the last decade, much progress has been made in growing these pluripotential hematopoietic stem cells in long-term in vitro cultures. These cultures allow maintenance and proliferation of pluripotential stem cells in vitro and also encourage differentiation and proliferation of committed progenitor cells that can be studied by the use of clonal assays. This long-term culture system has been applied primarily to studies of murine hematopoiesis, but it is currently being applied with increasing success to studies of human hematopoiesis.

Biochemistry and Function of Erythropoietin. EP is one of several hematopoietic growth factors that has apparently been purified to homogeneity from the urine of anemic humans. It is a glycoprotein (10 to 15 percent sialic acid) with a molecular weight of about 36,000. Approximately 50 to 60 percent of the molecule is carbohydrate, and it is not yet known whether the carbohydrate or the protein is the portion recognized by EP receptors on its target cells. Although several of the biochemical characteristics of the EP molecule have been defined, its exact structure has not yet been identified. There is overwhelming evidence that the major site of EP production is the kidney and that the control of EP secretion is effected through the oxygen tension of the blood in the kidney capillaries; the regulation of the biogenesis of erythropoietin, however, is incompletely understood. Research has shown that EP is a physiological regulator of erythropoiesis, but there are no definitive explanations concerning its mode of action.

Other Regulatory Factors. The defining of the requirements of growth of progenitor cells in clonal assays has led to research on the identification of humoral factors other than EP that may function in the regulation of erythropoiesis. One such factor, designated as burst-promoting activity (BPA), has been functionally identified and partially characterized. BPA most likely acts on early progenitor cells (BFU-E) and presumably enhances the viability of these cells. It is not yet clear whether this factor is one molecule or a family of related molecules, and there is no evidence that it has a regulatory function in vivo. Other factors

that are presumably involved in the regulation of pluripotential stem cells have been functionally identified in cell cultures of murine pluripotential stem cells.

Cellular Interactions. The concept that nonerythropoietic cellular elements may have important functions in the regulation of erythropoiesis has been advanced as a result of in vitro studies as well as of studies of congenitally anemic mice. The importance of marrow stromal cells in sustaining proliferation and renewal of pluripotential stem cells has been strongly suggested by studies of irradiated animals and congenitally anemic mice and by the establishment of long-term cultures. Evidence for a regulatory function of subsets of lymphocytes has been provided by in vitro experiments as well as by studies of mice with congenital defects in hematopoiesis. Evidence has also been presented that monocytes and macrophages have regulatory functions in erythropoiesis. Though extensive experimentation has suggested the importance of cellular interactions in erythropoiesis, progress in this field has been slow, mostly because of the inadequacy of currently available methods of experimentation with erythropoiesis.

Erythropoietin Distribution Program. The DBDR has supported a program that distributes large quantities of well-characterized human erythropoietin for use in studies of erythropoiesis. This program has significantly stimulated research in erythropoiesis by providing erythropoietin to a large number of investigators in the United States and around the world.

Accomplishments Through 1982: Abnormal Erythropoiesis

Aplastic Anemia

Aplastic anemia is a protean disorder of the bone marrow that is characterized by a reduction in the number or function of hematopoietic stem cells (HSC). It can be congenital or acquired, can vary from mild to fatal, and can affect one, two, or all three marrow-derived hematopoietic cell lines. An inadequate production of erythrocytes, granulocytes, and platelets can result. Most cases are acquired, and etiological factors include drugs, chemicals, toxins, radiation, and infection by viruses (frequently non-A,non-B hepatitis), pregnancy, paroxysmal nocturnal hemoglobinuria (PNH), and thymoma. Aplastic anemia is a serious illness, and until recently, it was associated with a 75 percent mortality rate. Progress in the field of transplantation biology and advances in the knowledge of the pathophysiology of this syndrome may change this gloomy picture. Therapeutic modalities have been developed that have somewhat decreased the mortality rates and have resulted in the cure of a few patients.

Bone Marrow Transplantation. The transplantation of hematopoietic stem cells in the form of bone marrow transplantation has emerged as the treatment of choice for patients with aplastic anemia who have a histocompatible donor. Prospective studies have defined the criteria for use of bone marrow transplantation and have demonstrated that pretransplant transfusions of blood from persons related to the marrow donor significantly reduce the chance of a successful transplant. Long-term survival studies have demonstrated the usefulness of bone marrow transplantation. Major improvements have been made in the management of graft versus host disease, which is the most severe complication of this therapy.

Supportive Care. Supportive care of patients with aplastic anemia has been improved by increased and more judicious utilization of blood components (red cells, platelets, granulocytes).

Increased Understanding of Aplastic Anemia. Investigators have obtained important insight into the etiology of aplastic anemia. In vivo and in vitro studies have provided evidence that one of the causes of aplastic anemia may be an abnormal hematopoietic microenvironment. In addition, abnormal interactions between erythroid progenitor cells and T lymphocytes have been observed. Observations suggesting that aplastic anemia may result from immune suppression of hematopoiesis include the demonstration of cytotoxic antibodies during the illness and the recovery of autologous marrow after treatment with immunosuppressive agents administered in conjunction with an unsuccessful bone marrow transplantation. In other patients, there seems to be a constitutional susceptibility to toxic agents.

Other Therapeutic Modalities. Since the majority of the patients with aplastic anemia cannot be treated with bone marrow transplantation because they have no compatible donor, many other therapeutic modalities have been investigated. Treatment to restore hematopoiesis with androgens, corticosteroids, lithium, cytoxan, and antithymocyte globulin (ATG) has been evaluated. By far the most encouraging results have been with ATG therapy. Favorable results in patients treated with ATG alone or in combination with other modalities have been reported. In a current randomized prospective trial of patients with moderate to severe aplastic anemia, 6 of 14 patients receiving ATG have improved, in contrast to none of 16 control subjects ($p = 0.04$). Confirmation of these data would suggest ATG treatment in patients who are not candidates for bone marrow transplantation.

Pure Red Cell Aplasia

There are several conditions in which erythropoiesis fails but in which other hematopoiesis apparently continues normally.

These conditions are referred to as pure red cell aplasia (PRCA). In these states, the marrow is normally cellular but is devoid of developing red cells. This condition can be either acute or chronic and also acquired or congenital.

Adult Onset Pure Red Cell Aplasia. This unusual disease, which occurs in adults, is characterized by acquired erythroid hypoplasia. It is frequently associated with thymic tumors, lymphomas, systemic lupus erythematosus, and other autoimmune diseases. In most but not all patients with adult onset PRCA, serum inhibitors (usually IgG) are present. These antibodies have been variously directed against erythropoietin, erythroblasts, CFU-E, or BFU-E.

Transient Erythroblastopenia of Childhood. Children with acquired red cell aplasia have a disorder called transient erythroblastopenia of childhood (TEC). Unlike adults, these patients improve rapidly. The sera of children with this disorder contain IgG immunoglobulin inhibitors of erythropoiesis. In a few cases, CFU-E have been found to be normal. Since TEC often follows a viral illness and interferon has been shown to inhibit erythropoiesis in vitro, interferon may have an in vivo importance.

Congenital Red Cell Aplasia. Congenital red cell aplasia, or Diamond-Blackfan anemia, is usually diagnosed in infancy. Serum EP levels are very high, and there is no evidence for a soluble inhibitor. The numbers of marrow CFU-E and BFU-E remain close to zero, and the number of circulating BFU-E is absent in relapse but remain low in remission. Currently, it appears that this disease can result from numerous etiologies, and studies suggest that there are defects in differentiation of erythroid progenitor cells or in the interaction between these progenitor cells and their environment.

Fanconi's Anemia

Constitutional aplastic anemias comprise one-third of childhood aplasias, and 75 percent of those are due to Fanconi's anemia. This is an autosomal recessive disease associated with physical anomalies and increased chromosomal breaks in which pancytopenia begins during childhood. No serum or cellular inhibitors of hematopoiesis have been found. Of these patients, 10 to 20 percent develop acute myeloid leukemia. The mechanism(s) is unknown by which the two mutant genes cause the disease and lead to pancytopenia. There may be an abnormal DNA breakage or repair mechanism, as suggested by studies of sister chromatid exchange, and abnormal characteristics of cycling of the cells. Recent studies suggest that oxygen metabolism is abnormal in Fanconi's cells. The relationship of these abnormalities to the in vivo stem cell defect(s) remains obscure.

Polycythemia Vera

Polycythemia vera disease is characterized by the production of too many red blood cells and is frequently accompanied by an overproduction of white blood cells and platelets. In studies utilizing G-6-PD markers, this condition has been demonstrated to result from a clonal stem cell disorder. Using in vitro assays of erythropoiesis, studies of the regulation of growth of the abnormal and normal cells have provided evidence that lines of normal cells continue to be present in the marrows of patients with this disease but that the proliferation and differentiation of the normal cells are arrested by an unknown mechanism.

A series of clinical studies conducted by a national polycythemia vera study group has established diagnostic criteria and has provided much information on the natural history of the disease and on the results of various therapeutic regimens.

Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria is another disease that results from a clonal disorder of the myeloid stem cell. It is characterized by an abnormal interaction of the membranes of the blood cells with activated components of serum complement. This abnormal interaction results in excessive breakdown or lysis of the cells, particularly red blood cells. The release into the bloodstream of large amounts of hemoglobin, which then passes into the urine, results; hence, the name of the disease. Extensive studies of this disease have provided insight into abnormalities of the blood cell membranes and their relationship to the complement system.

State of Knowledge in 1982

It is now clear that the regulation of hematopoiesis is a very complex process. The parent cells, which are the pluripotential hematopoietic stem cells, are known by their ability to populate hematopoietic tissues and by the functional properties of their progeny. It is not known how these cells become differentiated and committed to a particular cell line or how they sustain their potential for self-renewal. Furthermore, the factors that they require in order to proliferate and the type of cells that are required to aid them in their growth, renewal, and differentiation are not known. Culture techniques introduced during the last decade, which allow the study of stem cell proliferation, continue to provide further insight. These techniques have helped to clarify the complex processes of differentiation and maintenance of the numbers of different stages of progenitor cells, and

have helped in understanding the function of erythropoietin in the control of erythropoiesis.

Many of the requirements for growth and proliferation for various stages of erythroid progenitor cells have been defined. A serum-free medium has been devised in which some erythroid progenitor cells may be cultured. It has been demonstrated that erythropoietin has a very important function in the terminal maturation of older committed erythroid progenitor cells (CFU-E) and has relatively little influence on the growth and maturation of early committed erythroid progenitor cells (BFU-E).

The purification of erythropoietin has permitted the development of very sensitive radioimmune assays for erythropoietin.

There has been increased understanding of the interactions of the developing red cells and the microenvironment of the bone marrow. Although far from being fully explained, it is clear that there is a very specific interaction between the bone marrow and the growing and developing red cells. All of these events have provided further insight into the pathogenesis of aplastic anemia and the other myeloid stem cell disorders.

Three classes of erythroid progenitor cells and many aspects of their maturation have been defined. The mechanisms by which differentiation is achieved are entirely unknown. It is known that factors such as erythropoietin and burst-promoting activity are important in the process of differentiation of committed erythroid cells. Although EP has been purified, the chemistry of these factors and the mechanism of their action are not known. It is likely that factors other than EP and BPA have important functions in the regulation of erythropoiesis. Evidence indicates that "helper" or "managerial" cells may be involved in the regulation of erythropoiesis, but the identity of these cells and the mechanism of cell-to-cell interactions remain speculative.

In most cases of aplastic anemia, it appears that the marrow has a reduced number of pluripotential stem cells, although serum or cellular inhibitors of erythropoiesis have been implicated in a few cases. In general, marrow failure can be caused by the absence of, or defects in, hematopoietic stem cells, abnormalities of the bone marrow microenvironment, ineffective cell-to-cell interactions, and immune disorders. Evidence in experimental animal systems and clinical observations also suggest a possible function of certain viruses. Treatment of aplastic anemia involves blood transfusions and withdrawal of potential causal factors.

The value of prophylactic administration of platelets in the nonbleeding thrombocytopenic patient has not been proven, and the

use of prophylactic granulocyte transfusions in aplastic anemia is controversial. Efforts to stimulate hematopoiesis with androgens, steroids, and other drugs have been largely unsuccessful. Some patients may recover after treatment with antithymocyte globulin or other immunosuppressive agents. The mechanism by which ATG effects recovery of marrow, however, needs to be defined. Bone marrow transplantation has emerged as the preferred treatment for patients with severe aplastic anemia who have an HLA-identical sibling donor. Since the majority of patients do not have an HLA-identical sibling donor, alternative approaches are needed. Infusion of hematopoietic stem cells obtained from alternative sources, such as fetal liver cells or stem cells from long-term in vitro cultures, are promising approaches that need further exploration.

Abnormal erythropoiesis has been studied in murine as well as human models. A stem cell lesion has been defined in one form of murine congenital anemia (W/W^v) and a microenvironment defect in another (Sl/Sl^d). Studies of these models continue to provide insight into abnormal erythropoiesis in humans.

In summary, studies during the last decade have greatly increased the understanding of erythropoiesis. The concept of the process has changed from a simplistic one, in which erythropoiesis requires only erythropoietin-responsive cells and their regulator (erythropoietin), to the more realistic recognition that erythropoiesis is a complex, biologic process involving many cells and various factors of growth and regulation. Although the insights into disorders of the process have increased tremendously during the past decade, the conceptual methodological advances during that time have produced more questions than answers. Answers to these many new questions are essential to understanding more fully the disorders of erythropoiesis and to deriving effective treatments.

Needs and Opportunities

- As a result of the progress of the last 10 years, the significant complexity of the processes that regulate hematopoiesis (erythropoiesis) is recognized, but much remains to be learned. The protagonist cells (stem cells) are known only through the functional properties of their progeny and their ability to populate the hematopoietic tissue. Basic questions regarding the cellular and molecular events controlling hematopoiesis (erythropoiesis) remain largely unanswered.

- How stem cells become committed, how their self-renewal is sustained, what factors they respond to, and what type of

cells help in their proliferation, renewal, and commitment are matters of speculation. With the introduction of new technologic capabilities, such as techniques for maintaining stem cells in culture for long periods, gene cloning, cell sorting, and monoclonal antibodies derived from hybridomas, opportunities now exist for research on some of these complex interactions.

- Although the existence of the various classes of progenitor cells is apparent from the results of biological assays, the research on these cells during the last decade has been inferential. Certain characteristics of the progenitors have been investigated, but pure populations of the cells have not yet been isolated. It is now clear that further progress in understanding erythropoiesis and the mechanisms of differentiation will require isolation of pure populations of specific stem cells and the development of methods that allow their in vitro proliferation and differentiation. Efficient methods for separating and culturing primitive stem cells and erythroid progenitors are needed.

- Although the functions of erythropoietin and burst-promoting activity in the process of erythroid-committed cell differentiation are known, their chemistry and mechanism of action are not known. It is likely that factors other than erythropoietin and burst-promoting activity have an importance in the regulation of erythropoiesis, and it is possible that "helper" or "managerial" cells are involved in that regulation. Exactly what these cells are and what happens in cell-to-cell interactions remain speculative. Basic studies to delineate the mechanism of action of effector molecules and the nature of these cellular interactions and their genetic control should be encouraged. Such studies might be applicable to understanding such disorders as aplastic anemia, pure red cell aplasia, and polycythemia vera.

- Because of technical problems, little progress has been made so far in the application of culture technologies in clinical medicine. Yet it is certain that in vitro methods will be of importance for diagnosis, prognosis, and management of disorders of erythropoiesis. Practical application of culture techniques should be encouraged when the assays have been improved and standardized.

- The clues obtained during the last decade about interactive defects and immunological abnormalities in aplastic anemia and pure red cell aplasia should be pursued. Studies of congenital hypoplastic anemias should be

afforded high priority for funding since the mutants are expected to reveal the presence of regulatory events whose detection may be difficult with studies of normal cells. The in vivo and in vitro investigation of stem cell and clonal disorders that produce abnormalities in erythropoiesis should continue, particularly for preleukemic syndromes, paroxysmal nocturnal hemoglobinuria, and polycythemia vera. In contrast to malignant disorders, the understanding of the cell biology of these disorders is advanced, and efforts to delineate their pathogenesis may lead to significant basic advances in understanding and managing malignancy in humans.

Program Goals 1982 to 1987

Normal Erythropoiesis: Cellular

- Purify the various classes of stem cells and erythroid progenitor cells.
- Improve in vitro culture systems in order that progenitor cells can be grown in entirely defined (serum-free) media.
- Develop probes that allow the investigation of the regulation of erythropoiesis at the molecular level.
- Develop methods for the production of large numbers of pluripotential stem cells for therapeutic purposes.
- Define the type of cells interacting with stem cells or erythroid progenitor cells, and delineate the mechanisms of these interactions.

Normal Erythropoiesis: Humoral

- Purify and biochemically characterize the well-established erythropoietic factors, such as erythropoietin and burst-promoting activity. Define the mode of action of these factors and delineate their biogenesis and physiological action.
- Search for new factors involved in the regulation of erythropoiesis.
- Insure that the research community has access to high quality culture grade erythropoietin, and increase production of chemically pure EP.

- Develop established hematopoietic cell lines that can be used in the study of expression and commitment of differentiation, and establish and distribute cell lines that produce burst-promoting activity and other defined cellular growth factors.

- Develop a radioreceptor assay, and study the number of erythropoietin receptor sites on erythropoietin target cells.

- Use erythropoietin in affinity chromatography to isolate cells with erythropoietin receptors.

- Attempt to establish cell lines that are capable of producing erythropoietin or of responding to it in a physiologically understandable way. If a cell line that produces erythropoietin can be found, it would greatly accelerate studies utilizing genetic engineering to produce large quantities of the hormone.

- Study the mechanism of the generation and metabolism of erythropoietin in response to physiologic needs for red cell production. These studies should employ only refined and sensitive assays for erythropoietin such as the radioimmunoassay.

- Correlate the physiology of in vitro erythroid colony-forming cells with in vivo observations of erythropoiesis.

Abnormal Erythropoiesis

- Define the interactions between hematopoietic progenitor cells and the cells of the marrow microenvironment, and define the effects of different marrow toxic agents and humoral factors on different classes of hematopoietic progenitor cells in normal and disease states.

- Further elucidate the etiology(ies) and pathophysiology of aplastic anemia, and devise improved treatment for aplastic anemia. Encourage collaborative efforts with other NIH Institutes that actively support research on aplastic anemia.

- Delineate the cell biology, etiology, and pathogenesis of acquired and hereditary disorders of erythropoiesis.

- Support clinical studies designed to delineate the natural history, define the risk factors, and evaluate the therapeutic modalities in acquired and congenital disorders of erythropoiesis.

- Define the cellular defect that results in polycythemia vera, and elucidate the reason(s) for excessive thrombotic and hemorrhagic tendencies.

- Improve the understanding of the etiology, pathogenesis, and treatment of disorders such as refractory anemia, Di Guglielmo's syndrome, acquired sideroblastic anemia, and paroxysmal nocturnal hemoglobinuria. A fundamental need in the investigation of these disorders is the definition of the abnormalities of the product cells and the nature of the clonal proliferation and its control.

- Investigate the therapeutic efficacy of suitable erythropoietin preparations in selected disorders such as the anemia secondary to chronic renal failure.

Research Activities 1982 to 1987

Stem Cells and Progenitors

The currently available assays of erythropoiesis are of limited value since they do not allow direct studies of progenitor cells. Much controversy exists about the mechanisms by which erythropoiesis is regulated, about types of progenitor cells, and about the importance of cell-cell interactions and the cells involved in these interactions. It is now clear that further progress in the understanding of erythropoiesis will depend on obtaining probes that will permit the study of progenitor cells and stem cells. This research will require isolation of these cells from all the other cells present in the hematopoietic tissue. Research in this area should be supported with high priority.

Growth Factors

Humoral Erythropoietic Factors. Further progress in understanding of erythropoiesis requires that the two well-recognized factors EP and BPA become available in pure form. The slow progress in purification of factors and the inconsistencies in quality and composition of available preparations have provided major obstacles to in vitro experimentation on progenitor cells.

Additional Unrecognized Factors. Studies that demonstrate the importance of cellular interactions in erythropoiesis provide evidence for the existence of other factors, or activities, which may have regulatory roles. It is likely that these interactions are mediated through effector molecules that need to be defined.

Biological Action of Erythropoietic Factors. Little is known about the biogenesis of EP and even less is known about the physiological significance of BPA. If pure factors become available, this objective can be partially achieved with the use of existing progenitor cell assays, but ultimate definition of the action of factors will also depend upon the availability of populations of pure progenitor cells.

Therapeutic Potential of the Factors. Empirical studies in this area should be discouraged. Clues about therapeutic possibilities should emerge from physiological studies and investigations of the pathogenesis of disorders of erythropoiesis.

Research in Cultures of Stem Cells and Progenitor Cells

The first two new research activities listed above are necessary for devising scientifically reliable approaches to culture studies of stem cells and progenitor cells. It is likely, however, that the first of these two goals will require long and tedious experimentation. In the interim, efforts to improve current culture methodologies should receive high priority. Emphasis should be placed on the improvement of long-term cultures of human hematopoietic cells. These cultures are of relevance to all research focused on delineating the pathogenesis of disorders of erythropoiesis and also to research on the use of gene therapy for the treatment of disorders of hemoglobin synthesis and structure.

Study of Regulation of Erythropoiesis

Most of the current knowledge about the regulation of erythropoiesis is inferrential and indirect, and most of the current concepts of differentiation and regulatory events are hypothetical. Until purified factors and pure suspensions of defined progenitor cells become available, all experimentation for testing hypotheses will be indirect. Research on the regulation of erythropoiesis using currently available methodology should be supported, for it is expected that it will provide new clues to insights and new bases for research.

Investigation of Disorders of Erythropoiesis

The suggested importance of immunological abnormalities in aplastic anemia should be investigated. Studies of congenital hypoplastic anemias should be supported with high priority since the mutants would be expected to reveal the presence of regulatory effects whose detection might be difficult with studies of normal

cells. The in vivo and in vitro investigations of the clonal disorders that produce abnormalities in erythropoiesis should continue. Compared to knowledge of most malignant cells, the knowledge of the cell biology of these erythroid clonal disorders is highly advanced, and further work leading to the definition of their pathogenesis may have significant impact on the general understanding and handling of malignancies in humans.

Application of In Vitro Assays in Clinical Medicine

Because of current technical problems, little progress has been made in the application of culture technologies in clinical medicine. Yet, it is certain that in vitro methods will be of importance for the diagnosis, prognosis, and management of disorders of erythropoiesis. Practical application of the culture technologies should be supported once the assays have been improved and standardized.

The Circulating Red Cell

The red blood cell membrane, which is a cellular "envelope" with remarkable properties, consists of a series of specialized proteins that are inserted in, or applied to, a lipid matrix. A complex series of interactions among the proteins and between the proteins and the lipids provides the red cell with highly selective barriers, effective containments, and extraordinary strength and permeability. Modifications that alter these tightly coordinated interactions directly influence membrane function and, hence, cell survival. Several primary hemolytic disorders are known, such as the various hereditary spherocytoses, elliptocytoses, and stomatocytoses that involve derangements of these interactions, as well as the secondary hemolytic consequences of exposure to certain oxidant and peroxidant drugs, certain bacterial infections, malarial infestations, and various toxic chemical compounds.

The study of normal red cell metabolism has yielded a significant amount of basic information applicable to clinical problems. Disorders of red cell metabolism encompass both hereditary and acquired enzymopathies and acquired abnormalities. Hereditary enzymopathies can be further divided into the following major areas: hexosemonophosphate shunt and related aspects of glutathione metabolism, glycolysis, and nucleotide metabolism.

In the immune hemolytic disorders, red cells are destroyed by immunological processes--that is, by the interaction of antibodies and complement with the cell membrane. When antibodies are

attached to antigens on red cells, they signal that the cell should be cleared from the body by a set of reactions usually involving phagocytic cells or other cells able to mediate destruction (effector cells). Antibodies can arise in response to foreign antigens such as drugs (heteroantigens), to antigens of human red cells not possessed by the patient (alloantigens), and to antigens of the patient's own red cells (autoantigens). Such antibodies mediate red cell destruction by a number of mechanisms and facilitate the interaction between the target cell and the effector cells of the immune system. This interaction results in phagocytosis, partial phagocytosis, or cytotoxicity. The antibodies can also activate the components of complement, which are a group of proteins present in the serum in inactive form that, when activated, can either by themselves destroy the red cell (direct cytolytic action) or facilitate the interaction between the target cell and the effector cells of the immune system (opsinization).

Hemolytic anemias affect a large number of individuals. About 13 percent of black males in the United States have G-6-PD deficiency, and they are at risk for drug-induced hemolysis. Other inherited red blood cell enzyme deficiencies and red blood cell membrane defects are uncommon, but for those affected, particularly during early childhood, the utilization of medical resources is disproportionate to their number. Acquired red blood cell metabolic disorders are common, such as those associated with alcoholism and chronic kidney failure. Immune hemolytic anemia due to antibodies to heteroantigens or autoantigens is also a relatively common defined clinical problem. Treatment itself, while frequently effective, results in morbidity. Hemolysis due to antibodies to alloantigens, as seen in incompatible transfusion, remains a serious problem. It limits successful transfusion of red cells in some patients. Details of these problems are discussed in section 5 ("Blood Resources").

State of Knowledge in 1972

Red cell membrane research was in its formative stages in 1972. The analysis of red cell membrane lipids was fairly well-defined, and some of the pathways for lipid renewal had been elucidated. Very little was known about the the proteins of the membrane. Spectrin was the only red cell membrane protein that had been characterized to any appreciable extent. None of the interactions between the membrane proteins had been described, and the concept of the membrane skeleton had not yet been developed. Limited information concerning the surface charge of the membrane and considerable information concerning the characteristics of its permeability were available. Little was known about its deformability or fragility. Developments in both scanning and transmission electron microscopy, especially in freeze-fracture

techniques, had enabled advances to be made in the morphologic assessment of the membrane, although integration of this new information with biochemical or physiologic characteristics of the membrane was very rare.

Understanding of normal and abnormal red cell metabolism, particularly of specific enzyme deficiencies, was advanced. Variant forms of G-6-PD had been characterized, and severe deficiencies of several glycolytic enzymes had been identified. The vast majority of congenital, nonhemoglobinopathic hemolytic syndromes, however, remained undiagnosed. The basic science of immune hemolysis was established by 1972, and methodology for immunochemical studies were available. There was an advanced understanding of the complement system and of antibody structure and function.

Program Goals Through 1982

- Determine properties of red cell enzymes and their structure-function relationships.
- Determine control mechanisms in red cell glucose metabolism.
- Study the structure and function of the red cell membrane.
- Establish reference laboratories to provide standards for red cell enzymes.
- Prevent acquired disorders by removing the inciting cause. Identify drugs that cause hemolysis. Improve enzyme assays, and emphasize screening of the ill patient who might be at risk for complications of drug therapy.

Accomplishments Through 1982 and the Current State of Knowledge

Red Cell Membrane

Structure and Function. Progress in understanding the organization and the functional importance of red cell membrane lipids has been achieved. The asymmetric transmembrane distribution of lipids in the bilayer of normal cell membranes has been defined, and probable derangements of this distribution in selected disease states have been observed. Measurements of both the lateral and transmembrane motions of red cell membrane lipids by various probes, bleaching techniques, and magnetic and spin-resonance techniques have been accomplished. Several of the

limited, but probably functionally important, pathways of exchange and renewal for the maintenance of red cell membrane lipids have been elucidated. Qualitative and, to some extent, quantitative, analysis of many of the proteins inserted within, or adherent to, the lipid matrix of the red cell membrane have also been performed. The function of certain of these proteins (such as the anion transport capacity of band 3) has been defined.

Skeletal Proteins. The important concept of the skeletal proteins as the structural framework of the membrane has been developed. Research to elucidate the functional interactions within these skeletal proteins (such as the functions of spectrin, ankyrin, actin, band 4.1) and their various interactions in providing membrane shape and integrity has been initiated.

Ion Regulation. Progress has been made in understanding the regulation of ion movements by membrane components, including the function of various channels and pumps. The importance has been appreciated of the capacity of the membrane to prevent calcium ingress for protecting the cytoplasmic functions and perhaps the function of the membrane itself.

Technical Developments. Sensitive gradient gel procedures and radioimmunologic techniques have been refined and permit the analysis of extraordinarily small amounts of peptide components of the membrane; new methods for measuring physical properties and whole cell deformability have been developed; chemical probes and physical methods (such as electron-spin resonance, nuclear magnetic resonance, and fluorescence depolarization) as well as biochemical and enzymatic probes have been applied to the study of intact red cell membranes; and the highly sensitive assays have been developed for receptors of various agonists within red cell membranes.

Clinical Implications. Clinical achievements that have resulted from developments in both the basic understanding of the red cell membrane and the technical capacity to study the membrane include an understanding of the membrane lipid abnormalities that occur in severe liver disease and acanthocytosis (spur cell anemia); an appreciation of the clinical importance of the mean cell hemoglobin concentration in several conditions characterized by indeformable cells (in particular, sickle cell disease); the definition of the importance of particular proteins in various disorders of cell shape and deformability (hereditary elliptocytosis, pyropoikilocytosis, and some cases of hereditary spherocytosis); and the suggestion that the red cell membrane may be representative of underlying membrane abnormalities in important somatic conditions, such as neuromuscular dystrophies, hypertension, and obesity.

Red Cell Metabolism and Enzymopathies

Regulation of Glycolysis. There has been an increase in knowledge of the normal regulation of glycolysis and of the enzyme diphosphoglycerate mutase of the Rapoport-Luebering shunt pathway. Erythrocytic enzymes, such as phosphofructokinase and pyruvate kinase, have been characterized.

Genetic Heterogeneity. A significant degree of genetic heterogeneity of red cell enzyme defects has been found. More than 150 different mutant G-6-PD's and a large number of mutant pyruvate kinases are now recognized, and standard procedures have been developed for characterizing pyruvate kinase variants.

Nature of Defects. The majority of red cell enzymopathies have been shown to be due to structural gene mutations. Gene products are usually present, although they are sometimes unstable or catalytically inactive. A small number of mutant G-6-PD's and phosphoglycerate kinases have been partially sequenced, and amino acid substitutions have been identified.

Clonal Origins of Tumors. Sex-linked enzymes (G-6-PD) identified through studies of red cells have been employed as markers for investigating the multicellular or unicellular origin of tumors, including leukemias, polycythemia vera, and paroxysmal noctural hemoglobinuria. In addition, an apparently clonal origin of certain atheromatous plaques has been demonstrated.

Delineation of Diseases. New glycolytic and hexosemonophosphate (HMP) shunt enzymopathies have been delineated. Hemolytic anemias that result from severe deficiencies of hexokinase, glucosephosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, phosphoglycerate kinase, and pyruvate kinase are currently recognized. Red cell enzyme deficiencies (such as a severe deficiency of diphosphoglycerate mutase), which can influence the function of the red cell as a carrier of oxygen by affecting the concentration of red cell 2,3-diphosphoglycerate, have been detected. New red cell disorders due to abnormalities of enzymes of purine and pyrimidine metabolism have been described. Pyrimidine-5'-nucleotidase deficiency has been identified as a cause of hereditary hemolytic anemia associated with unique accumulations in red cells of pyrimidine ribonucleotides, and the investigation of pyrimidine-5'-nucleotidase has provided insights into the mechanism by which the normal reticulocyte rids itself of the products of ribosomal breakdown as it matures. The enzyme was found to be exquisitely sensitive to inhibition by lead. In subjects with severe lead intoxication, inhibition of pyrimidine-5'-nucleotidase results in accumulations of red cell pyrimidine and hemolytic anemia. An unusual syndrome in which

adenosine deaminase (ADA) is significantly increased (40- to 75-fold) has been identified, and this finding has provided additional insights into adenosine metabolism in humans. This enzymopathy reinforces evidence of a role for adenosine in salvage pathways for crucial adenine ribonucleotides. The introduction of a new adenine-containing blood preservative solution (CPDA-1) represents a major practical advance that can directly be attributed to an increased understanding of nucleotide metabolism in red cells.

Biopsy Material. The red cell has been used increasingly in the diagnosis of several nonerythrocytic disorders, including galactosemia, oroticaciduria, Lesch-Nyhan syndrome, riboflavin deficiency, immunodeficiency associated with ADA or nucleoside phosphorylase (NP) deficiencies, and hepatic porphyrias.

Enzyme Replacement Therapy. Use of red cells for enzyme replacement therapy has been initiated. An accumulation of deoxyribonucleotides in lymphocytes is a major abnormality in immunodeficiency due to deficiency of ADA or NP. Theoretically, the accumulation of these metabolites can be diminished by transfusing with erythrocytes that have normal ADP or NP so as to reduce the concentrations of deoxyribonucleotides accumulated in the blood. Clinical studies testing this possibility have been started, and preliminary reports appear promising.

Immune Hemolysis

Red Cell Antigens. Considerable progress has been made in the definition of antigens of the red cell surface with which antibodies interact. A clearer definition has been made of serological specificity of autoantibodies. The biochemical characterization of red cell antigens, particularly those with polysaccaride determinants, has been refined. Details of the ABO system and the relationship of this system to the P and I-i system have been defined. The antigens such as M, N, S, s, U, T, and Tn associated with membrane glycoproteins have been identified, and the understanding of the chemical structure of these molecules has been advanced.

Antibody Structure. Significant advances have been made in understanding the structure of antibodies and the relationship of their structure to the destructive processes. The interaction of cell-bound antibody with receptors on effector cells (Fc receptors) and the function of the various effector cells, particularly lymphocytes, have been elucidated.

Biochemistry and Function of Complement. Considerable progress has been made in understanding the biochemistry of the

reactions of complement and the importance of these reactions in the immune destruction of red cells. Details of complement-mediated immune adherence, including the knowledge of the receptors for C3 and C4 components of complement on the effector cells, have been elucidated. The mechanism of the penetration of the membrane by the membrane attack complex (C5b-9) of complement has been investigated, but the details are still lacking.

Hemolytic Disease of the Newborn. Work on the eradication of hemolytic disease of the newborn by prophylactic administration of hyperimmune anti-D began in the 1960's. It is now clear that, with appropriate prophylaxis, this clinical problem can virtually be eliminated. This is a major milestone in the control of genetic disease. Approximately one case of serious and often permanent illness for every 1,000 live births has been eliminated by this simple but effective clinical maneuver.

Paroxysmal Nocturnal Hemoglobinuria. Progress has been made in understanding the hemolysis in paroxysmal nocturnal hemoglobinuria, which is a disease characterized by an abnormal membrane that interacts in an unusual way with components of complement.

Technical Improvements. Improved techniques for the qualitative and quantitative detection of molecules of antibody and complement on the red cell, including the use of specific antisera for IgG and its subtypes and for specific complement components, have enhanced the ability to identify specific causes of immune hemolysis. The relation of these measurements to the rate of destruction of the cells and to the response to therapy has been, in part, elucidated.

Program Goals 1982 to 1987

Overall goals with regard to the research on the red cell membrane should include:

- Foster a more complete understanding of the development, structure, function, and senescence of the normal membrane. This knowledge should make it possible to improve therapy for membrane disorders.

- Utilize the red cell as a tissue for diagnosing other diseases. The study of the regulation of ion exchange by the membrane, the regulation of pathways of lipid exchange, and the regulation of cell surface receptors appear to be three areas where red cells may be useful for this purpose.

Overall goals with regard to the research program in red cell metabolism and enzymopathies are:

- Advance the understanding of normal and abnormal red cell metabolism and enzymology with special emphasis on errors of red cell metabolism.

- Characterize normal and variant red cell enzymes at the molecular level; define the significance of acquired enzyme aberrations; and elucidate the critical metabolic factors determining the life span of the erythrocyte.

- Determine the mechanism of hereditary and acquired disorders that have diminished red cell survival, in order to develop adequate therapy.

Overall goals with regard to the research program in the immune hemolytic anemias are:

- Improve the basic knowledge of the structure of the red cell membrane so that immunological reactions can be more fully understood.

- Improve the understanding of the reactions that lead to the production and control of causative antibodies.

- Improve the understanding of the control of cellular and humoral mechanisms, with special emphasis on immune hemolytic anemias.

Research Activities 1982 to 1987

Red Cell Membrane

- The contribution of the membrane to red cell survival needs to be elucidated. In particular, the relative importance of flexibility of the membrane and of its other physical properties, its ion barrier role, its geometric distribution, and its antigenicity need to be established.

- The understanding of the membrane skeleton and of the relative importance of the various interactions between skeletal proteins needs to be established. The function of the several remaining uncharacterized proteins found in the red cell membrane needs to be established.

- The senescence of the red cell and the mechanisms for its eventual recognition and destruction should be elucidated.

The role of membrane lipid and protein oxidation and peroxidation, as well as the senescence of membrane enzymes and the possible change in membrane antigenic components, should all be considered in this general area.

- How the special shape of the red cell is preserved should be elucidated, and the biophysical importance of that shape in normal red cell function should be further specified. It should be determined how phospholipid asymmetry is established and maintained across the membrane bilayer.

- The significance of modifications such as glycosylation and phosphorylation of membrane proteins should be elucidated. In addition, regulation of phosphorylation and how the regulation is influenced by somatic disorders and the red cell milieu need to be studied.

- The mechanisms for the coordinated assembly of membrane proteins of the red cell should be determined. The synthesis and insertion of membrane proteins into the lipid matrix, and cellular maturation and nuclear extrusion, for example, are important events that need clarification for understanding the evolution of the reticulocyte.

- The relation of protein and lipid composition to membrane shape and to physical properties should be determined. Such information should allow for studies focused on modifying membrane components for treating the various membrane pathologic states.

- The possible use of the red cell membrane as a model and diagnostic tool in other diseases should be explored, such as hypertension, muscular dystrophy, and obesity, as well as other potential receptor-mediated, membrane protein and membrane lipid disorders.

- Studies should be encouraged for identifying and isolating the genes responsible for the synthesis of membrane proteins. Such investigations will be valuable for identifying and potentially ameliorating diseases.

- Efforts should be made to centralize the development and distribution of monoclonal antibodies against various membrane polypeptide constituents. Centralization would provide an economical and efficient means for advancing research on the red blood cell membrane.

- The development of methods employing cell fusion, liposomes, or osmotic exchange for modifying or replacing membrane components should be encouraged.

- The development of animal models for specific membrane disorders should be encouraged, such as shape and ion-transport abnormalities, membrane-receptor abnormalities, and systemic disorders.

Red Cell Metabolism and Enzymopathies

- In spite of excellent progress in the past decade, many hematopoietic syndromes, including those producing hereditary and acquired anemias, are not yet pathogenetically defined. Continued investigation of congenital hemolytic anemia is required in order to define the pathogenesis of hemolytic anemias of unknown etiology and elucidate the many different mechanisms by which a defective enzyme can exert deleterious effects.

- There is as yet only minimal information regarding the whole process of production, maintenance, and decay of red cell enzymes and of the critical metabolic factors that assure red cell survival for the normal 120 days. Improved understanding of this basic area is clearly needed, and it has great practical importance for blood preservation.

- The function of the hexosemonophosphate shunt in erythrocytes needs to be further defined. Increasing use of drugs with an oxidant effect on red cells and other tissues gives this research a clinical relevance. The effects of acquired disease, systemic disorders, and drug therapy on red cell metabolism should be investigated.

- Investigation should be encouraged of the use of red cells as a biopsy tissue for the diagnosis of inherited or acquired metabolic disorders. This research would be very useful since the red cell, unlike the cells of most tissues, is safely and easily obtained and readily separated from other cell types.

- Basic research on the biochemistry and molecular biology of red cell enzymes and metabolism should continue. Expanded knowledge in the area of red cell metabolism will inevitably lead to a basic understanding of normal metabolism and regulatory mechanisms not only of the erythrocyte but also of body tissues that utilize the same or similar metabolic pathways.

- Efforts should be encouraged to evaluate and determine the benefit of selective screening of populations at risk for G-6-PD deficiency. Several high-risk populations can be readily identified, such as persons entering the health care system who are very likely to be given drugs that might produce hemolytic anemia or to develop infections that may also precipitate hemolysis in G-6-PD deficient individuals. Additionally, individuals who are traveling abroad and require antimalarial prophylaxis may be candidates for screening for G-6-PD deficiency. Some investigators have already begun to routinely screen patients for G-6-PD deficiency who may be seeking hospitalization for various reasons. This screening, however, has not been done on a sufficiently large scale or with sufficient followup to enable an assessment of the ratio of cost to benefit. If large-scale screening is found to be cost-efficient, it would provide a simple method for preventing and protecting many people from developing severe and, in rare cases, even fatal reactions to certain oxidant drugs such as primaquine, sulfonamides, and furadantin.

Immune Hemolysis

- A better understanding of the processes that initiate the autoimmune hemolytic syndromes is clearly needed. In particular, the reasons for the production of an antibody against an antigen of self are still unclear. The important progress made in basic immunological techniques should be applied to research focused on understanding the undoubtedly complex and heterogeneous causes of autoimmune hemolysis. In particular, the relationship to viral diseases and to other disorders of the immune system including neoplasms needs to be elucidated.

- Techniques for the detection of immunological reactions of the red cell remain imperfect. The clinically available direct Coomb's test is inadequate in defining the syndrome for approximately 1 patient in 40 with immune hemolytic anemia. The problem is greater in transfusion therapy where many hemolytic reactions to transfusion cannot be detected beforehand by usual techniques. Research should be strongly encouraged to improve methods.

- The understanding of the basic reactions and intercellular events that lead to cellular destruction and phagocytosis is incomplete. Additional research should be encouraged in this area.

- Research on the control of the production of antibody is needed. At the present time, therapy for autoimmune

hemolysis is too nonspecific. Specific means of treatment, such as better drugs and allotypic suppression, are clearly needed, along with additional research on the therapeutic control of the reactions of complement.

Hemoglobin

Fundamental research on hemoglobin has had a special impact in biology and medicine. It has provided insights and produced the prototypes for understanding human genetic disease in general, and it has a direct relevance to the delineation, management, and eventual therapy of two common hereditary diseases, sickle cell anemia and Cooley's anemia. There has been a significant investment during the last 30 years in fundamental research on hemoglobin, and this investment has produced handsome results. Fundamental hemoglobin research has also had an impact on the practice of medicine. The delineation of the pathophysiology of sickle cell anemia and Cooley's anemia has resulted in progress in their treatment, prenatal diagnosis, and prevention. Hemoglobin research has also had an important impact on other fields of medicine by providing models for, and understanding of, human disease in general.

State of Knowledge in 1972

By 1972, several aspects of the genetics of the hemoglobin genes had been worked out. It was known that there exist alpha-like genes (the zeta and alpha loci) and beta-like genes (the epsilon, gamma, delta, and beta). It had been established that alpha- and beta-like genes are not linked and that gamma, delta, and beta genes are linked. The basic biochemistry of hemoglobin and the importance of primary, secondary, and tertiary structures for hemoglobin stability and function were well appreciated. Functional studies and x-ray crystallography had identified specific parts of the hemoglobin molecule that are of crucial significance for the function of hemoglobin as a carrier of oxygen. Over 150 abnormal hemoglobins had been identified and structurally analyzed, and the analysis of certain of these mutants was providing additional insights into the normal processes of oxygen transport. By 1972, the existence of aberrations in hemoglobin ontogeny that can lead to continued production of fetal hemoglobin (Hb F) in adults, and the therapeutic importance of the synthesis of increased amounts of fetal hemoglobin in patients with sickle cell anemia and in certain forms of thalassemia were fully recognized. Work on the biosynthesis of hemoglobin subunits was at an advanced level. Methods existed that allowed preparation of globin messenger RNA (mRNA), and progress had been made in

utilizing globin mRNA in cell-free systems to analyze the factors that regulate the translation of globin mRNA's.

Program Goals Through 1982

The goals of fundamental research set in the 1972 National Program were:

- Understand, on the molecular level, the role of each amino acid residue in oxygen binding and release, and the interaction of hemoglobins with intracellular molecules that regulate oxygen transport.
- Determine structure and function of hemoglobin variants.
- Determine the function of chemically modified hemoglobins.
- Explore immunochemical methods for identification of hemoglobins.
- Develop specific gene therapy: isolation or synthesis of hemoglobin genes.
- Study transcriptional and translational control of globin chain synthesis imbalance.
- Elucidate mechanisms of gene switching, specifically the switch from fetal to adult hemoglobin.

Accomplishments Through 1982

There has been striking progress in the pursuit of these goals. Developments that could not have been predicted in 1972 occurred in the mid-1970's, and the achievements that resulted surpassed even the most optimistic projections. The field of molecular biology of hemoglobin was catapulted by advances in recombinant DNA technology. The globin genes now provide a prototype for investigation of the organization and regulation of other genes in eukaryotic cells.

Hemoglobin Biochemistry and Physiology

Investigations of hemoglobin continued to develop new biomedically relevant information. The three-dimensional structure of human hemoglobin was refined to very high levels of resolution (2 angstroms), and the information provided important insights into the mechanisms that underlie the physiologic behavior of hemoglobin. The structural information was supplemented by a

large and authoritative body of spectroscopic data, such as nuclear magnetic resonance, electron paramagnetic resonance, resonance Raman, and infrared and fluorescence spectroscopies. New information was acquired on the environment around the heme group as well as on the alterations in protein conformation that accompany ligand binding. The interactions of the two most important physiologic regulators, hydrogen ions and organic phosphates (2,3-DPG), were delineated at all levels from structure to physiology. The composite body of structural and spectroscopic information was applied to the formulation of allosteric and related models that explain the functional properties of hemoglobin. The large amount of information on hemoglobin helped to delineate the behavior of other hemoproteins and multi-subunit enzymes.

A few specific advances can be cited. A large and internally consistent body of information on the thermodynamics and kinetics of interactions of hemoglobin subunits at various stages of oxygenation became available. The physiologic mechanism of methemoglobin reduction was delineated, and the reducing agent and its enzyme were thoroughly characterized. Hemoglobin was shown to be useful as a reporter molecule, reflecting environmental fluctuations in health and disease. Measurement of Hb A_{1c}, formed by the glycosylation of hemoglobin, was found to be useful in monitoring the control of hyperglycemia in diabetic patients, and it provided insights into the pathogenesis of long-term complications of diabetes. It was also found that other posttranslational modifications of hemoglobin take place as a result of renal failure and alcohol abuse.

Molecular Biology

Protein Synthesis. Mpch of the knowledge of protein synthesis has come from the use of the reticulocyte as a model system. The central role of eukaryotic initiation factor-2 (EIF-2) in initiation of protein synthesis has been established. This protein and other factors involved in eukaryotic protein synthesis have been purified. It has been shown that the activity of EIF-2, which is affected by oxidation and phosphorylation, provides a means for cells to control protein synthesis in relationship to other metabolic requirements.

Organization of Globin Genes. The chromosomal localization of the human alpha-like and beta-like gene complexes has been defined. The position of the individual genes within each complex has been identified in humans as well as in animals such as mice, rabbits, chickens, goats, and sheep. Globin pseudogenes, which are homologous to functional genes but inactivated by mutation, have been discovered. Evidence has been obtained that these pseudogenes are found within the globin gene cluster and are also dispersed throughout the genome. Members of several repetitive

DNA families have been identified within the globin gene clusters and have been characterized structurally. They have provided information that allows formulation of specific experiments to determine their function.

Structure, Function, and Evolution of the Globin Genomic Regions. The definition of the exact nucleotide sequences of many globin genes is leading to an emerging concept of the canonical globin gene. It was discovered that coding parts (exons) of the gene that specify the structure of a globin protein are separated into three blocks by two intervening sequences of DNA (introns). The entire gene, including the introns, is copied in mRNA, but the intervening sequences must be removed to generate a functional mRNA. Consensus sequences important for efficient processing of mRNA have been identified, and their functional importance has been verified by study of varíous mutations that cause thalassemia. The promoter, namely the site at which RNA polymerase binds and gene transcription begins, has been defined by sequence analysis. Model systems designed to study transcription in cell-free extracts and within living cells have verified the functional significance of the promoter sequences. Knowledge of the DNA sequences that immediately flank the globin genes has provided insight into globin gene evolution that results from gene deletion, gene duplication, and intrachromosomal exchanges.

Structure of Chromatin at the Globin Genomic Regions. The DNA in animal cells is associated with histones and other proteins in a nucleoprotein complex called chromatin. The basic component of chromatin structure, namely the nucleosome, has been defined. Studies using mainly the systems of nonhuman globin genes have shown that the particular nonhistone chromosomal proteins HMG14 and HMG17 are associated with the nucleosomes on expressed globin genes, where they establish an active conformation that allows these genes to be transcribed into mRNA. Certain small segments of DNA near expressed globin genes are exquisitely sensitive to nucleases. Therefore, these segments appear to lack nucleosomes and thus may serve as sites for binding of proteins that regulate chromatin structure and gene expression. Monoclonal antibodies have been obtained that react specifically with nonhistone proteins present in red cells of embryonic and adult chickens. Such proteins may be involved in gene regulation. The relationship between modification of DNA at the globin genomic regions and the expression of globin genes has been explored. It has been found that compared to most DNA, the DNA of expressed globin genes is relatively undermethylated. This finding suggests that the absence of methyl groups may be necessary for formation of the active chromatin conformation.

Development of Globin-Gene Transfer Technology. Alteration of the genetic complement of living cells has been accomplished by insertion of new genes. The genes and their surrounding DNA

sequences obtained by molecular cloning have been introduced into living cells by calcium-phosphate-mediated DNA transfer, by direct microinjection, and by viral vectors.

Requirements for globin gene function have been defined by the introduction of modified or mutated genes into cells to study their function. Such studies have allowed the promoter to be characterized. By using a combination of recombinant DNA technology, molecular cloning, and screening of recombinants for the relevant gene by expression of such genes after their transfer into animal cells, new strategies for purification of genes have been formulated and tested.

New and unanticipated information about gene function and regulation has been obtained by these approaches. The mouse globin gene promoter, for instance, has been shown to function in a mouse erythroid cell line in culture, but the human gene promoter does not. This unexpected species specificity provides an approach to define the components of the gene that allow regulation to occur. Also, when DNA is injected directly into fertilized eggs, it is incorporated into the genome of the resulting animal. Preliminary data suggest that such genes are expressed in specific tissues and that normal regulation of these genes has been achieved during development. Thus a new approach has been provided for investigating the basis for such developmental regulation. In addition, retroviruses have been shown to act as a vehicle for highly efficient gene transfer into tissue culture cells. By incorporating only the portion of the retrovirus genome that allows for viral packaging and integration into host cell DNA along with a globin gene, investigators might be able to prepare recombinant viruses that can be used for efficient transfer of globin genes into hematopoietic stem cells without risk of transformation.

Hemoglobin Switching

Progress has been made in understanding the regulation of human fetal hemoglobin synthesis in vivo. Immunochemical methods were developed for detection of fetal and adult hemoglobins in single cells. Studies of patients with clonal hematopoietic stem-cell disorders supported a common stem-cell origin of Hb F-producing erythrocytes (F cells) and of the erythrocytes without Hb F expression. Production of F cells was associated with acute erythropoietic expansion after bone marrow transplantation or after recovery from transient hypoplastic anemia or chemotherapy. Genetic influences in the production of F cells were detected.

Analysis of hemoglobin synthesis in clonal erythroid cultures was used initially to study the switch from adult hemoglobin (Hb A) to hemoglobin C in the sheep model and subsequently to study the hemoglobin synthesis in colonies derived from human cells.

Commitment to express specific genes appears to occur during maturation of stem cells and progenitor cells although the proportion of Hb A and Hb F is also modulated during maturation of erythroblasts. Several factors have been shown to affect the proportion of Hb A and Hb F synthesized in individual colonies, but whether these factors exert direct effects on gene expression or alter Hb F synthesis as a secondary consequence of progenitor selection or perturbation of the maturation process is unknown. Commitment of progenitor cells to form erythroblasts that synthesize Hb F appears to occur in a stochastic manner and to be regulated at a stage in the erythroid pathway prior to the initiation of hemoglobin synthesis. Evidence has been obtained that the perinatal switch from Hb F to Hb A is regulated at a much earlier stage in the erythroid pathway, perhaps in the pluripotential stem-cell compartment.

Animal models have been developed for studying the cellular regulation of Hb F in vivo. It has been found that induced acute anemia results in the stimulation of the production of fetal hemoglobin in adult baboons. Treatment with 5-aza-cytidine exaggerates the Hb F response. The sheep model has been used extensively in searching for in vivo factors that may function in switching. Transplantation experiments were used to determine whether the regulatory mechanisms are intrinsic to the stem cells and reflect a biological "clock" or whether the stem cells respond to stimuli in the changing microenvironment and alter the hemoglobin phenotype of their progeny erythroblasts. A few successful experiments have been done in the sheep model, but the results are not yet definitive.

Human cell lines were developed that are useful for the study of the cell biology of human hemoglobin switching. Two human erythroleukemia lines, the K562 and the HEL cells, display induced erythroid differentiation. Their significance is that they express embryonic and fetal hemoglobin but not adult hemoglobin. Studies have been initiated that focus on characterization of these cell lines.

State of Knowledge in 1982

The structure and organization of the human globin gene complexes are now defined, and the DNA sequence of each gene has been determined. The coding block of each gene is interrupted by two introns that are transcribed along with coding sequences to yield a precursor RNA, the processing of which must include precise removal of intervening sequences to yield mature mRNA. The factors required for efficient translation of globin mRNA have been purified, and regulation of translation is an active area of investigation. Various mutants of the globin genes have provided clues to the nature and location of DNA sequences that effect gene

expression. Several in vitro strategies for introducing functional genes into cells provide an experimental approach for directly testing the regulatory importance of specific DNA regions. Clonogenic assays for erythroid progenitors have been used to investigate developmental hemoglobin switching. The mechanisms that regulate the expression of particular globin genes within the alpha or beta globin gene complex are operative in erythroid progenitors. The perinatal switch from fetal to adult hemoglobin production appears to reflect a biological clock intrinsic to stem cells or, alternatively, reflect the influence of instructive hematopoietic microenvironmental factors on the behavior of progenitor cells.

Program Goals 1982 to 1987

- Support research on organization and expression of globin genes with emphasis on development of functional assays and on studies of chromatin.
- Search for a more physiologic and practical means of globin gene transfer.
- Simplify the molecular technology used in diagnostic procedures.
- Define the molecular and cellular mechanisms that are responsible for the switch from Hb F to Hb A formation during ontogeny and for the activation of fetal hemoglobin synthesis in the adult.
- Delineate the molecular dynamics of the function of normal and mutant hemoglobins.
- Support research in assembly, function, and degradation of hemoglobin in the erythrocyte.

Research Activities 1982 to 1987

Molecular Studies

- Continued support is required for research related to gene structure and function. The vast amount of information accumulated about gene structure must be supplemented as other regulatory mechanisms are being identified. Current functional assays are not adequate. New techniques are particularly needed for investigating regulatory mechanisms. Identification of those chromosomal proteins

involved in gene regulation may eventually allow reconstitution of the chromosome in vitro and creation of a transcriptional complex that is as fully activated and modulated as it is in vivo.

- Future support is required for research directed to developing more physiological and practical gene transfer. Introduction of genes into cells can now be achieved in vitro for analysis of their function, but the goal of genetic therapy can be achieved only if genes are selectively introduced and selectively expressed in target cells in vivo with high efficiency.

- Techniques for defining genetic defects by gene analysis using restriction endonucleases and DNA sequencing are now available in highly specialized research laboratories. Simplification of this methodology is essential if it is to be useful at the clinical level for prenatal diagnosis and characterization of genetic disease. The financial incentive provided by the potential widespread use of this technology may stimulate commercial and industrial interest, which will possibly reduce or eliminate the need for direct support of this applied research effort. In the interim, support is recommended for one or two "reference" laboratories to confirm the accuracy of prenatal molecular diagnoses of sickle cell anemia and thalassemia.

Hemoglobin Switching

- Continued support is required for research related to the delineation of the molecular basis of human hemoglobin switching. Investigations should continue on the molecular heterogeneity of mutants associated with the persistence of fetal hemoglobin synthesis into adult life. DNA sequencing and chromatin studies of such mutants should be encouraged. The molecular basis of expression of fetal and embryonic globins in human erythroleukemia cell lines needs to be clarified. It is anticipated that the molecular studies of mutants and the development of more efficient functional assays will allow recognition and functional characterization of those DNA sequences that regulate the switching of globin genes.

- In vivo and in vitro research should continue on the cellular mechanisms of switching. The animal models developed during the last decade should be fully utilized. Studies should continue on phenomenology in human patients, for human phenomenology is always a source of information for which animal models cannot be substituted. The information about stem cell regulation of hemoglobin

switching obtained during the last decade through the application of culture technology needs to be studied in more detail. The question of intrinsic versus interactive control of Hb switching during ontogeny needs to be solved, and the exact mechanism by which the adult cell can change its phenotype and produce fetal hemoglobin needs to be determined. Means should be explored for the in vitro and in vivo induction of Hb F synthesis in adult cells with the objective of developing procedures that will be harmless to cells and to the individual. To a large degree, progress in understanding the cellular regulation of hemoglobin switching depends on new experimental advances in erythropoiesis. Research focused on characterization and isolation of progenitor cells, identification of the cellular and humoral factors that control differentiation and erythroid progenitors, and the development of new and more efficient progenitor and stem-cell culture methods should be supported. These latter developments are also required for in vitro experimentation with gene transfer.

Biochemistry and Physiology of Hemoglobin

- Extraordinary progress has been made in the last decade in understanding the structure and function of the hemoglobin molecule. The next frontier is the study of the basic physics of hemoglobin and involves the use of very high resolution techniques combined with other approaches such as statistical mechanics, thermodynamics, and molecular dynamics. Specifically, very high resolution x-ray crystallography of normal and mutant hemoglobins, high resolution spectroscopic studies of hemoglobin and ligand binding, and very fast kinetic studies of the mechanism of ligand binding are required. These approaches will allow a description of the mechanism of ligand binding in terms of very fast processes (picoseconds) and involve very small molecular distances and binding energies. A detailed picture of the interactions between oxygen, the solvent, and the hemoglobin molecule may result. These studies will provide a truly quantitative understanding of the normal function of hemoglobin in the red cell and the diverse ways that this function is influenced by normal physiological variables and in many diseases.

- Most of the progress in understanding the physiology of hemoglobin has come from studies of relatively dilute, cell-free solutions. Much remains to be learned about how hemoglobin actually functions in the very nonideal environment inside the erythrocyte. It is important that the process of assembly of the hemoglobin tetramer from globin

and heme be understood. The interactions of hemoglobin with other molecules in the cell should be explored at the very high hemoglobin concentrations that exist in the red cell. The chemical changes and eventual degradation of hemoglobin should also be defined in molecular detail. Specific research areas, particularly in relation to such diseases as thalassemia, hemolytic anemias, diabetes, uremia, and malaria, should include: assembly of globin and heme in the cell; interactions of hemoglobin with ligands, effectors, enzymes, and other cellular constituents; degradation of hemoglobin at various stages in the life of the erythroid cell; and modification of hemoglobin during the life span of the erythrocyte. These studies should provide information about the normal function of hemoglobin as well as changes that affect its function in disease.

Sickle Cell Disease

Sickle cell anemia (Hb SS disease) is one of the most common hereditary disorders among people of African descent. In the United States, approximately 1 of 500 black newborns is homozygous for the hemoglobin S gene, and 1 in 12 black adults has the sickle cell trait and is at risk for parenting a child with sickle cell anemia. Since in the U.S. black population Hb C is common (1 in 33 has the Hb C trait) and beta-thalassemia is found with a frequency of 1 percent, a significant number of patients with Hb C/Hb S disease and Hb S/beta-thalassemia exists. The sickle cell syndromes appear infrequently among other Americans, except for those of Greek, Italian, and Middle Eastern ancestry.

The disease was first described in this country in 1910 by Dr. James Herrick, and the "molecular" defect was identified in the early 1950's. Sickle cell anemia is caused by the presence of an abnormal hemoglobin molecule due to substitution of valine for glutamic acid at position 6 of the beta chain. Deoxygenated sickle hemoglobin is less soluble than normal hemoglobin (AA) and aggregates upon deoxygenation to form intracellular polymers that distort the red blood cell into a sickled shape. Sickle cells are rigid, and as they traverse the microcirculation, they tend to occlude small capillaries. The occlusion prevents oxygen from reaching tissues and results in chronic organ damage. In contrast, normal red blood cells are pliable and traverse small capillaries without difficulty. In sickle cell disease, the life span of these cells is abnormally shortened, and chronic anemia can be a consequence.

The common clinical manifestation of the sickling syndromes is the occurrence of crises, which consist of episodes of debilitating pain lasting up to several days. The major pathology noted in sickle cell patients living into adulthood is the gradual deterioration of organ function produced by microcirculatory obstruction. Crises and organ damage lead to frequent hospitalizations. In addition, psychosocial problems mount as patients grow into adulthood. The problems are aggravated by the fact that little effort has been directed toward effective ways to solve them.

State of Knowledge in 1972

Sickle cell disease had been shown to be due to a point mutation of the hemoglobin molecule, beta-6 Glu Val. Single crystals of oxy Hb S analyzed in 1951 yielded patterns of diffraction that were indistinguishable from those obtained from oxy Hb A. Hence, the two structures appeared to be the same. However, for the more important deoxy form, x-ray data were lacking, since the available microcrystals were unsuitable for x-ray study. Electron microscopy showed the polymeric aggregate of deoxy Hb S molecules to consist of rod-like structures with parallel arrangements, each with a diameter of approximately 200 angstroms. The technique, however, was not sufficiently sensitive to demonstrate the structural details of these rods.

Increased amounts of fetal hemoglobin (Hb F), heterogenously distributed within the red blood cells, was a well-known characteristic of the disease, and there was considerable evidence that sickle cell disease was positively modified, in its clinical expression, by increased levels of intracellular Hb F. This evidence came from studies of individuals heterzygous for both the sickle gene and the gene for hereditary persistence of fetal hemoglobin as well as from specific populations of sickle cell patients, such as Saudi Arabians, with unusually high levels of hemoglobin F and only mild symptoms of disease. These clinical studies were consistent with data derived from gelation experiments that demonstrate that increasing amounts of fetal hemoglobin increase the minimum gelation concentration (MGC). These studies were also consistent with differential centrifugation studies showing that sickle cells rich in Hb F survive longer than the cells with predominantly sickle hemoglobin. The pattern of Hb F production in humans and in some primates during development was well characterized.

In the early embryo, there is a group of embryonic hemoglobins that are replaced during development by Hb F (gamma chains). The Hb F is replaced by Hb A (beta chains) during the first postnatal year. The structures of the peptide chains of the human hemoglobin are directed by separate gene loci. The non-alpha chain genes were thought to be linked in the following

order: G gamma, A gamma, delta, beta. Studies of patients with hereditary persistence of fetal hemoglobin suggested that deletion of critical areas of this gene cluster is responsible for persistent gamma-chain synthesis in adult life.

It was known that the activity of the gamma-chain loci is almost entirely "turned off" during the first postnatal year, but that a small amount of Hb F production persists and is limited to relatively few (about 3 percent) red cells. A variety of genetic disorders were known in which Hb F production persists and Hb F reappears in several acquired conditions. The acquired conditions include pregnancy, some forms of leukemia, and the aplastic anemias, as well as after bone marrow transplantation. These observations indicated that the gamma chain genes are not entirely repressed in adult life and can be reactivated in response to a variety of environmental changes.

There was virtually no experimentation before 1972 on the mechanism of hemoglobin switching, largely because of the lack of good experimental models. The only model that had been studied in detail was the erythropoietin-induced switch of Hb A to Hb C in sheep and goats, but this model had only limited application to the "switching" mechanism of human fetal hemoglobin.

Available diagnostic techniques to identify abnormal hemoglobins were primarily limited to hemoglobin electrophoresis and to the demonstration of sickling by use of agents that remove oxygen. The presence of large amounts of fetal hemoglobin at birth made early diagnosis an impossible task, and most infants with sickle cell disease before 1972 were undiagnosed until after 6 months of age. The establishment of federally funded screening programs with quality control monitored through the Center (now Centers) for Disease Control (CDC) has led to accurate diagnostic programs with appropriate education, counseling, and followup. Prenatal diagnosis, however, was still not possible.

In 1966, hydrophobic interactions between the N terminal valine and beta-6 valine were thought to have a major function in the polymerization of deoxyhemoglobin S. Since urea presumably disrupts hydrophobic bonds, clinical studies were undertaken to assess its efficacy through a controlled double-blind study supported by the NIH. Parenteral urea was shown to be ineffective in the treatment of sickle cell crises. Other therapeutic approaches included alkali, steroids, and anticoagulants, all of which were proven to be ineffective. The most promising agent, cyanate, was shown to have an antisickling effect both by increasing oxygen affinity and decreasing polymerization. Some patients improved after oral administration of cyanate, but later, the development of cataracts and neurotoxicity proved it to be too toxic. The unacceptability of these side effects and the potential benefits of cyanate led to investigations to develop a system

to deliver cyanate by the extracorporeal route. Animal studies were initiated, and a crude apparatus was constructed to evaluate the approach of treating red cells outside the body by removing the excess agent and reinfusing treated red cells. In the early 1970's, investigations in search of the "magic bullet" for sickle cell disease were accelerated. Current research has continued with a more systematic approach to drug development.

In 1972, the general public, patients, and many health care professionals had limited and often inaccurate information about sickle cell disease. Clinical management was often fragmentary and was punctuated by episodic care during acute events. As a "great mimicker," sickle cell disease affects almost every organ system, and symptoms of complications requiring immediate attention often went unrecognized. Premature deaths from overwhelming sepsis in early childhood and progressive organ damage prior to the second decade were accepted as the natural history of sickle cell disease. In the United States, approximately 25 percent of newborns with sickle cell disease died by the age of 4, many from treatable complications. The importance of infection in the morbidity and mortality at a young age and the high risk for fatal pneumococcal sepsis were clearly known.

The clinical spectrum of sickle cell anemia was historically reported as a very severe, time-limited disease. Textbook descriptions, which focused on the most debilitating symptoms, portrayed a skewed view for both patient and practitioner. This inaccuracy can be attributed to the limited knowledge of mildly affected patients, since patients who sought medical care in emergency rooms and those who were hospitalized represented the population that was most severely affected. The description in the scientific literature of the sickle cell trait as a "mild form" of the disease extended an ignorance about the clinical process.

Program Goals Through 1982

- Increase basic and clinical research, clinical applications, clinical trials, training and education, and efforts in screening, counseling, rehabilitation, and information dissemination.

- Improve the understanding of the molecular structure of sickle hemoglobin, interactions of other abnormal hemoglobins with sickle hemoglobin, differences in flow patterns between normal and sickle red cells, and the effect of various chemical agents on the sickling process.

- Develop simple and accurate techniques for the early clinical detection of abnormalities produced by sickle hemoglobin.
- Develop approaches to patient management based on the latest scientific advances.
- Develop improved therapy for sickle cell crises, and increase the knowledge of the fundamental biology of the disease and its complications.
- Develop effective and acceptable methods for screening and counseling.
- Define the natural history of sickle cell disease.
- Develop collaborative efforts with other Institutes of the NIH and other Federal agencies.

Accomplishments Through 1982

Biology of Sickling

Structure of the Sickle Cell Polymer. The architecture of the polymer of Hb S was investigated in order to obtain information that might allow designing ways of inhibiting the formation of polymers. Studies of mixtures of hemoglobins in solutions and the elucidation of the crystal structure of Hb S have been achieved through the techniques of high resolution electron microscopy of the fiber, x-ray diffraction, and image reconstruction. The Hb S polymer was found to be a high-order arrangement of the double strand of molecules present in the crystal of Hb S twisted into a 14-strand cable. Many of the areas of contact between deoxy Hb S molecules were defined. This advance should permit the design of stereospecific reagents to serve as antisickling agents of potential clinical use.

Mechanism of Polymerization. New techniques have been established to study the rate and extent of formation of sickle cell polymers as a function of oxygen pressure, total hemoglobin concentration, temperature, pH, and fraction of other hemoglobins such as normal and fetal hemoglobins. These studies have led to a description of the "gel" of sickle hemoglobin as consisting of a solution phase with normal-oxygen affinity and a polymer phase of low-oxygen affinity. Because the hemoglobin in the solution phase occupies a large fraction of the volume, the solution is highly nonideal. Insights on the kinetics of formation of polymers were obtained. It was found that prior to the appearance of polymers,

there is a "delay time," which is sensitive to several physiological variables. The delay occurs because a finite period of time is required for the formation of nuclei prior to polymerization. Such a delay might permit cells to escape from the narrow capillaries before polymerization occurs. It is now thought that increasing the length of the delay relative to the period of capillary transit should ameliorate the disease. This understanding of the mechanism of polymerization has suggested the possibility of new therapeutic approaches including efforts to diminish the concentration of intraerythrocytic hemoglobin and thereby reduce intracellular polymerization.

Membrane Abnormalities in Sickle Cell Disease. Investigation of the membrane of sickle cells (Hb SS), initially stimulated by observations on irreversibly sickled cells (ISC's), has led to three developments: better information on transport functions of the membrane during sickling; detection of phospholipid rearrangement in SS cell membranes; and discovery of an abnormal adherence of SS red cells to vascular endothelium. All three developments have clinical importance. When SS cells sickle, C^{2+} ions enter and K^{+} ions and water leave the cell (the Gardos effect), and ultimately a pathologic elevation in mean corpuscular hemoglobin concentration (MCHC) occurs. Recent recognition of drugs that inhibit the Gardos effect carries therapeutic implications. Another membrane alteration that occurs in SS cells as they sickle is a rearrangement of phospholipids in the asymmetrical lipid bilayer in such a way that molecules with potentially pathologic procoagulant activity now appear at the cell surface.

Sickle Cell Rheology. With the use of new methods, studies have been conducted of morphological and filtration changes upon the kinetics of deoxygenation, of the viscosity and filtration of sickle cells under various physiological conditions, and of cell fractionation. An animal model of modified human sickle cells infused into rats has been proposed, and a primate model for infusion of sickle cells has been developed.

Fetal Hemoglobin in Sickle Cell Syndromes. The beneficial effect of elevated levels of fetal hemoglobin in sickle cell syndromes was further confirmed by in vitro kinetic studies and by studies of patients. It was also found that in erythroid cultures, erythroid cells of Hb S patients can produce Hb F that, if produced in vivo, would inhibit sickling.

Prenatal Diagnosis

Before 1972, prenatal diagnosis of sickle cell anemia was impossible. Initial efforts were directed to acquisition and direct analysis of the very small amounts of beta chains in fetal

blood samples obtained by fetoscopy. These efforts were successful. Prenatal diagnosis has been utilized only sporadically, however, by the black community. Among the reasons for this situation are: the cost of the procedure, social factors, and the availability of fetoscopy in only a handful of highly specialized clinics for prenatal diagnosis.

Major developments occurred with the introduction of molecular technology to prenatal diagnosis. A method of diagnosis based on new findings about restriction endonuclease polymorphisms allowed prenatal diagnosis in several, but not all, pregnancies at risk. This method also required similar genetic studies of members of the family for the interpretation of findings from the examination of amniotic cells. Spbsequently, efforts were made to develop restriction endonuclease-mapping technology to recognize the abnormal codon of the Hb S gene. This objective was accomplished, and the feasibility of this approach in prenatal diagnosis of sickle cell anemia was demonstrated.

The practical significance of this major development in sickle cell research in the past decade is overwhelming. Every pregnancy at risk for producing a child with sickle cell disease can now be monitored. Amniocentesis, a procedure that is widely available for obtaining cells for analysis, with very low risk to the mother or to the fetus, is alone required. It is now possible for prenatal diagnosis of sickle cell anemia to be available to every couple in the black community.

Therapeutic Approaches

In the 1970's, new concepts of treating sickle cell anemia were developed, in vitro experiments were conducted, and clinical trials of treatment by protein modification were initiated. Initially, urea was used as an antisickling agent because of the in vitro findings of its antisickling properties and of the claims of clinical improvement of patients in a sickling crisis. A cooperative study of several centers, however, failed to show therapeutic effects of urea. Subsequently, cyanate was introduced. Extensive biochemical studies in solution and in red cell suspensions provided strong evidence that hemoglobin carbamylation decreases the propensity of red cells to sickle. Initial pharmacological studies in animals failed to reveal deleterious effects. Treatment with oral cyanate was introduced in a clinical trial, and improvement of the hematological parameters and a decrease in the frequency of crises were reported. Controlled studies were initiated to test the therapeutic efficacy of oral cyanate, and they showed little effect on frequency of crises at the oral dosages of cyanate used. In addition, the detection of neurotoxic side effects dictated discontinuation of the use of oral cyanate in sickle cell anemia.

The experience with cyanate disclosed one of the problems raised by the approach of protein modification in the treatment of sickle cell anemia. Chemicals that modify hemoglobin can modify other vital proteins and lead to unwarranted side effects. To overcome the possibility of toxicity of orally administered agents with antisickling effects, alternative modes of administration were probed, and the possibility of extracorporeal delivery of such compounds was tested. A technique was developed for removing blood from the patient, treating it extracorporeally in a renal dialysis-type machine, dialyzing out the excess reagent, and reinfusing the blood into the patient. Testing for safety has been successfully conducted, and feasibility studies have been initiated.

During the last decade, the advances in understanding the structure of the polymer of deoxyhemoglobin S and the mechanism of its formation, including the functions of physiological variables, have allowed a whole range of biochemical approaches. These include the use of gelation inhibitors, such as covalent or noncovalent inhibitors of deoxyhemoglobin S contacts in gel; agents that decrease hemoglobin S concentration or increase oxygen affinity; cell-sickling inhibitors, such as membrane modifiers; and inhibitors of microvasculature entrapment. At this point, however, no agent has been developed that is known to be both safe and effective.

Assays of antisickling agents have been improved. The classical cell-sickling assays have been complemented, and in some ways supplanted, by assays of the thermodynamics and kinetics of deoxyhemoglobin S gelation, assays of the intracellular polymerization of deoxyhemoglobin S, and assays of the oxygen affinity of concentrated solutions of hemoglobin S and sickle erythrocytes.

During the last few years, an NIH-supported laboratory for a comparative screening of antisickling agents was established. With the use of the above assays, over a dozen of the major chemicals are being studied as potential therapeutic agents. This information should be an important point of reference in the development of future compounds.

Two laboratories have been funded to design, with the knowledge of the structure of the polymer, and to synthesize inhibitors of Hb S gelation. In addition, about five laboratories in the NIH intramural program or on NIH grants and several laboratories funded by pharmaceutical companies have active programs of drug development.

Clinical Studies and Patient Care

Diagnosis. Improved electrophoretic techniques using samples of cord blood have made it possible to diagnose sickle cell disease in the newborn. Identification of patients in early infancy allows immediate education and counseling of the parents about the risks of sepsis. This most devastating complication contributes to mortality and morbidity in this age group. The entry of the infant into the health care system prior to developing complications now increases the potential for reducing early morbidity and mortality. Physicians who are knowledgeable of this diagnosis can respond aggressively to potentially life-threatening infections.

Infections. Clinical investigations completed during the past decade firmly established the contribution of infections to morbidity and mortality of sickle cell patients. Although sepsis is a continued hazard in sickle cell disease, it has its highest impact in infancy and early childhood. It has been learned that pneumococcal septicemia in children with sickle cell anemia is the single greatest contributor to mortality through the age of 5. Functional hyposplenism may contribute to the increased susceptibility to bacterial infections. The development of a partially successful pneumococcal vaccine has reduced the impact of pneumococcal sepsis, though patients are still susceptible to gram-negative infection and are not entirely protected from pneumococci.

Transfusion. Improvements in transfusion technology have had a beneficial effect on sickle cell anemia. Recognition of marked differences in the risk of sensitization of blacks to minor blood groups that are absent in blacks and present in whites has improved approaches to blood typing for the transfusion of sickle cell patients. Introduction of frozen blood for chronic transfusion has reduced the risk of allosensitization from contaminating leukocytes that induce febrile reactions. Other improvements in transfusion practice include the introduction of the new hepatitis B vaccine, which protects patients with sickle cell anemia from one of the most pernicious hazards of blood transfusion, and the development of improved methods of recognizing cytomegalovirus (CMV) carriers in blood donors. Exclusion of such donors will reduce the hazard of acquired CMV infection in pregnant women with sickle cell disease and hence reduce the risk of CMV damage to the central nervous system of their offspring.

Ocular Complications. Progress has been made in the understanding of sickle cell retinopathy. The nonproliferative and proliferative ocular changes have been classified, and the evolution of proliferative sickle retinopathy into sequential stages from peripheral arteriolar occlusions to retinal detachment has been delineated. It was shown that argon laser or xenon arc

photocoagulation can effectively close neovascular tissue, but is associated with significant complications including ischemia, hemorrhage, and retinal detachment. A randomized trial has been undertaken to determine the efficacy of photocoagulation in proliferative sickle cell retinopathy.

Initiation of Cooperative Studies. A large-scale national cooperative study involving 23 institutions and 3,535 patients has been initiated to investigate the clinical course or natural history of sickle cell disease. The cooperative study is expected to extend the understanding of the clinical aspects and improve the management of sickle cell disease and to provide baseline information for future research, including evaluation of anti-sickling agents. Knowledge of risk factors, rates of progression, the incidence of complications, and the effect of sickle cell disease on the life of the patient will directly aid physicians in the evaluation and care of patients. Preferred treatments will emerge from an analysis of the outcome of complications of the disease. Accurate knowledge of the effects of the disease on the economic, educational, vocational, social, and psychological status of the patient will provide a basis for designing health care and social services for these patients.

Education and Screening. The development of sickle centers has led to a far greater understanding within the black community of the characteristics of sickle cell disease and to acceptance of the concept of screening for parents at risk. Educating both the public and the patient about sickle cell anemia and removing myths and misconceptions have led to greater participation in service programs for the entire community.

Comprehensive Care. One of the most important results of the creation of sickle cell centers has been the development of improved comprehensive management of patients with the disease. Such patients can have disorders of almost every organ system, and treatment in the past has tended to be fragmented and episodic. Through the center programs, a comprehensive team approach has emerged that employs highly trained physicians, nurses, and social workers capable of dealing with the entire spectrum of problems facing the sickle cell patient. This approach may have influenced appreciably the life span and quality of life of many patients.

State of Knowledge in 1982

- The overall structure of the polymer is now reasonably well established. Many of the structural sites involved in intermolecular contacts are known, and for some of the intermolecular contacts, there is a rather complete three-dimensional picture in atomic detail. It is now theoretically possible to systematically approach the

problem of blocking polymerization by an agent that is stereospecific for a site of intermolecular contact.

- The general features of the mechanism of polymer formation from purified solutions are understood in terms of a nucleation mechanism, which accounts for the period of delay, and a two-phase model for the resulting gel. Polymerization inside red cells most probably proceeds by a mechanism that is the same as the one in purified solutions. A variety of techniques are now available to study the rate and extent of gelation. The delay time of polymerization is very sensitive to physiological variables, and the length of the delay relative to the period of capillary transit may be a critical factor determining the relative severity of the various sickle cell syndromes. The understanding of the kinetics of sickling provides a basis for developing therapeutic approaches in sickle cell disease.

- Newborns can be accurately diagnosed for sickle cell disease with the use of samples of cord blood. Such a diagnosis allows for comprehensive management and surveillance of early symptoms of infections.

- The high incidence of bacterial meningitis and septicemia during the early pediatric age appears to be on the decline. The polyvalent pneumococcal polysaccharide vaccine has been partially successful in protecting the patient over 2 years of age from fulminating pneumococcal infections, and the vaccine has been incorporated into the management plan for patients over the age of 2. There continue to be serious infections from strains contained in the vacine, from other pneumococci, and from gram-negative bacteria. Prophylatic penicillin is also used by a large number of clinicians to prevent infections.

- A regimen of repeated transfusions, usually referred to as "chronic" transfusions, to prevent the recurrence of cerebrovascular complications in childhood have been accepted as appropriate therapy for this group of patients. The benefits of repeated transfusions in other chronic complications are less well established, although this regimen is frequently used for priapism, intractable crises, and pregnancy. Improved techniques for processing cellular components and automated systems for exchange transfusions make it practical to offer this therapy to a wide population of sickle cell disease patients. Transfusing younger red cells ("neocytes"), which survive longer than older cells, increases the interval between required transfusions, and it helps prevent iron overload.

Hepatitis, another complication of transfusion, can now be prevented with the new hepatitis vaccine.

- Couples with sickle cell trait now have the option for prenatal diagnosis of sickle cell disease through amniocentesis and molecular analysis of DNA from amniotic cells.

- Recent studies have suggested that interactions of alpha-thalassemia with sickle cell disease may mitigate the clinical impact (severity) of sickle cell disease by reducing the concentration of intracellular hemoglobin. The importance of hemoglobin concentration to the aggregation of sickle hemoglobin has led to clinical efforts to reduce the hemoglobin concentration in red cells by therapies that may be difficult for the patient to accept, such as dilutional hyponatremia.

Program Goals 1982 to 1987

- Support basic research especially in: polymer formation and structure, membrane structure and function, definition of methods for gene insertion into hematopoietic stem cells, induction of fetal hemoglobin synthesis, and development of suitable animal models to study human sickle erythrocytes in vivo.

- Investigate the basis of sickle cell deformability in the light of the biochemical and biophysical developments of the last decade.

- Support quantitative rheological studies of sickle erythrocytes in new filtration and microvasculature systems.

- Develop noninvasive methods for monitoring blood flow and microvasculature behavior in sickle cell patients. The relation of the SS cell to the microcirculatory dysfunction is a prime area to be studied.

- Support research on the design, development, and evaluation of new antisickling agents. A major goal is the development of effective, nontoxic therapeutic agents.

- Promote collaboration among government, university, and pharmaceutical institutions in the development of therapeutic agents.

- Improve clinical care and management of patients with sickle cell disease, including psychosocial aspects and better approaches to management of pain.

- Develop objective methods for diagnosing sickle cell crisis.

- Develop and test therapeutic modalities:

  - Develop an effective oral iron chelator

  - Assess the clinical efficacy of sodium cyanate by the extracorporeal route

  - Investigate the feasibility of bone marrow transplantation in sickle cell disease

  - Consider a long-term clinical trial of anticoagulation (coumadin).

- Continue support of comprehensive sickle cell centers and implementation of the center concept.

- Delineate the natural history through the cooperative study of sickle cell disease.

- Establish a program allowing utilization of the option of prenatal diagnosis and encourage further development and support of regional centers for prenatal diagnosis.

Research Activities 1982 to 1987

- Since a knowledge of the polymer structure is the foundation of research on the development of stereospecific inhibitors of polymerization, elucidation of the intermolecular contacts is essential. Further electron microscopic and x-ray diffraction studies are required to establish with absolute certainty the number and arrangement of the hemoglobin strands of the polymer in a variety of solutions. To determine the amino acid residues involved in the intermolecular contacts between double strands, further copolymerization studies are needed, particularly of alpha-chain mutants. The design of stereospecific agents will require elucidation of the structure of the intermolecular contacts in atomic detail.

- Future research on the mechanism of polymerization is needed in several areas. The kinetics of polymerization requires further study to understand more fully the mechanism of nucleation. High resolution nuclear

magnetic-resonance studies might be able to determine the intermolecular contacts that form first in the nucleation process. Time-resolved electron microscopic techniques might give information on the relation between polymer alignment and length and the rate of polymer formation. Very little is known about the growth of polymer domains. The size and structure of the domains could be very important in determining the rheological properties of gels, and hence the flexibility of the red cell. In general, kinetic studies could lead to new, therapeutically useful approaches to inhibit polymerization. A major effort should be made to study polymerization in single cells and in fractionated cells as a function of physiological variables such as oxygen pressure and hemoglobin composition. Comparative studies in cells from the various sickle cell syndromes should define quantitatively the inhibitory effects necessary to achieve a specified improvement in clinical status.

- Further investigation of the red cell membrane should yield two important advances in understanding and treating sickle cell disease: knowledge of the mechanism responsible for the interaction of the sickle cell membrane with the endothelial surface of capillary vessels, which may provide new avenues for therapeutic intervention; and the development of membrane-active drugs that lower intracellular hemoglobin concentration.

- Rheological research, which has not kept pace with progress in other aspects of sickle cell disease research, should be supported. Attempts should be made to improve mechanical models (such as viscosity instruments, nucleopore filters) to better simulate physiological conditions. Of equal importance is the use of new techniques such as lasers, ultrasound, and nuclear magnetic resonance to devise noninvasive ways of studying blood flow in sickle cell patients. These methods would allow much more sophisticated ways of evaluating potential therapies as well as provide insights into the cellular pathophysiology of sickle cell anemia.

- Support of work on antisickling agents should continue. Examples of areas that need effort include: stereospecific agents (with efforts centering on avoiding the modification of other proteins); safe and effective methods to diminish the intracellular concentration of hemoglobin by modifying the membrane or by altering favorably the Donnan equilibrium of the cell (through the introduction of osmotically active chemicals); potential use of liposomes to encapsulate active antisickling agents

and to deliver them exclusively to red cells; and development of simpler extracorporeal delivery systems that include the possibility of home use.

- During the past decade, there was significant progress in understanding fetal hemoglobin production in adults. Most promising were the observations that the erythroid cell both in vitro and in vivo can be manipulated to synthesize Hb F. If Hb F instead of Hb SS had been produced in the red cells of a patient with sickle cell disease, the potential for sickling would be avoided. This promising area of investigation should continue, with emphasis on understanding the cell biology of Hb F synthesis in the adult and on developing harmless approaches of induction of Hb F production in patients.

- Although gene therapy is in its infancy and experimentation is faced with significant difficulties, priority should be given to overcoming these difficulties and to determining safe and reliable vectors that will allow the introduction of normal genes by normal genes in stem cells that have sickle genes. This advance would represent a direct application of modern molecular biology in the treatment of sickle cell disease.

- Sickle cell crisis is the major acute clinical problem facing patients with sickle cell anemia. The pathophysiology remains elusive because the events that trigger painful crises are not clearly established. Research on the precipitating factors as well as the development of means of objective diagnosis should be supported with high priority.

- Studies of the natural history of sickle cell anemia should continue. What is the life expectancy of the victim? What is the incidence of crises? What organ systems are involved? Can the thrombotic manifestations be controlled or predicted? These are but a few of the enormously complex questions that can be resolved only by the continuation of an extremely important long-term followup program instituted 4 years ago by the NHLBI. It is imperative that such a program be continued.

- Certain therapeutic modalities need to be tested. There is a deficiency, for instance, in systematic long-term studies of transfusion treatment, and there is controversy about the management of pregnant patients. Among the alternative therapeutic modalities for sickle cell anemia, the transplantation treatment should be probed since bone marrow transplantation has been successfully applied in a wide range of disorders. Risk of the procedure is

currently much too high for use in a disorder with as long a life span as that held by most patients with sickle cell anemia. Improvements in the control of graft versus host disease and in the control of infection, and a better understanding of the long-term effects of the various drugs used in bone marrow transplantation may aid application of this technology to sickle cell disease.

- Sickle cell anemia behaves as a chronic vasoocclusive disease that is not inhibited by known anticoagulants as they are currently administered. Anticoagulants do not appear to change the incidence of painful crises. Whether long-term interruption of the synthesis of functional coagulant proteins by a drug such as coumadin will alter the chronic course of sickle cell anemia is not actually known. The effect of anticoagulant treatment on sickle cell anemia should be investigated. The development of radioimmunoassays capable of precise determination of inactive and active prothrombin molecules in plasma has shown that the determination of the level of factor II activity in plasma can precisely permit safe and reproducible anticoagulation. Long-term trials of coumadin therapy in sickle cell anemia from an early age should be considered as part of the ongoing cooperative study to determine whether the incidence of chronic complications can in fact be reduced by such a treatment program. Such a program should be implemented through the existing comprehensive centers.

- The techniques for prenatal diagnosis of Hb S disease are 100 percent accurate. It is recommended that: two or more laboratories be supported to provide cultured amniotic cells and restrictive endonuclease mapping; the existing screening and education programs that are conducted in centers and clinics be reevaluated on the basis of their ability and expertise to offer the option of prenatal diagnosis to counselees; and a national program that includes sickle cell centers, sickle cell clinics, genetic centers, and genetic clinics be instituted. The goal of this program should be to test methods for the delivery of counseling to the black community in combination with prenatal diagnosis. It is recommended that this program operate through existing services so that the major expenses be limited to those of amniocentesis, clinic fees, and the logistical expense of shipment of amniotic cell samples to the laboratories. Information about acceptability of prenatal diagnosis by the black community cannot be gathered unless prenatal diagnosis becomes widely available through a large variety of counseling and service outlets.

The Thalassemias

The thalassemia syndromes are hereditary hemoglobin disorders that result from diminished or absent synthesis of adult hemoglobin in red cells. There are two general types of thalassemias, those affecting the alpha genes of hemoglobin (alpha-thalassemia), and those affecting the beta genes (beta-thalassemia or delta, beta-thalassemia). An average of 5 to 7 percent of Americans of Mediterranean ancestry and approximately 1 percent of black Americans are carriers of the beta-thalassemia gene. Two carriers of beta-thalassemia are at risk for having offspring with Cooley's anemia (homozygous beta-thalassemia). A beta-thalassemia carrier and an Hb S carrier are at risk for having offspring with Hb S/beta-thalassemia disease. These persons suffer from chronic anemia and painful sickle cell crises.

Patients with homozygous beta-thalassemia die in infancy or childhood unless they receive frequent blood transfusions. As a result of repeated transfusions, excess amounts of toxic iron accumulate in vital organs. Such patients die as teenagers unless iron is removed by an iron-chelating drug.

A large segment of black Americans and persons of Mediterranean and Oriental ancestry are carriers of alpha-thalassemia genes; they are at risk for having offspring that present with the syndrome of Hb Bart's hydrops fetalis or Hb H disease. In Hb Bart's hydrops fetalis, affected infants are either stillborn or die within hours of birth. Hb H disease is characterized by chronic hemolytic anemia. The individuals with either beta- or alpha-thalassemia trait present with hematological abnormalities that are readily detected by routine hematological measurements.

The study of affected individuals has led, in recent years, to the accumulation of a vast body of knowledge about the molecular biology of normal and abnormal globin gene expression. This information is critical in the design of therapeutic approaches in the thalassemia syndromes.

State of Knowledge in 1972

By 1972, the various forms of thalassemia and related abnormalities, such as hereditary persistence of fetal hemoglobin, had been recognized and well characterized at the phenotypical level. Globin biosynthetic defects had been recognized in alpha- and beta-thalassemia syndromes. It had been postulated that the thalassemias are due to defects in messenger RNA synthesis or stability, and research had been initiated to define the defects of mRNA. The pathophysiology of the beta-thalassemia syndromes was well understood by 1972, and the deleterious effects of

imbalanced globin synthesis was appreciated. Hypertransfusion treatment protocols had been initiated, and it was recognized that a major risk for the patient with Cooley's anemia is iron overload resulting from frequent transfusions. Initial studies had been reported that showed beneficial effects of iron chelation treatment. The populations at risk in this country had become aware of the genetic consequences of thalassemia in their ethnic groups, and the initiation of screening and counseling programs were demanded. Procedures allowing diagnosis of the thalassemia traits, however, remained in the domain of specialized laboratories.

Program Goals Through 1982

The recommendations of the 1972 National Program were to:

- Determine the frequency of thalassemia in identified target populations of Mediterranean ancestry and their attitudes about mate selection.
- Determine frequency of thalassemia in blacks.
- Develop a plan to have a registery of patients with thalassemia major.
- Develop methods of prenatal detection.
- Develop better and less expensive screening tests.
- Determine an optimal transfusion program for thalassemia major.
- Improve methods for chelation and excretion of iron.
- Collect data on the natural history of thalassemia.
- Study transcriptional control of chain imbalance.
- Study translational control of chain imbalance.

Accomplishments Through 1982

Basic Research

In the mid-1970's, molecular studies on thalassemia focused on mRNA. Improved methods were developed for isolating globin mRNA from bone marrow and peripheral blood reticulocytes. In the

thalassemias, mutations associated with diminished or with absent globin mRNA were found. Cell-free systems were developed and used to study the function of the thalassemic mRNA.

The molecular pathology of alpha-thalassemia syndromes has been delineated further. Studies with structural hemoglobin variants have suggested that the alpha genes are duplicated in humans. Normal persons have four alpha-globin chain genes--two from each parent (alpha alpha/alpha alpha). The application of molecular hybridization techniques has provided evidence that the alpha-thalassemias in Orientals and blacks are due to deletions of one or more of the four alpha genes. The phenotypes of alpha-thalassemia syndromes have been correlated with the number of deleted alpha genes. Globin gene mapping of index cases of deletion mutations has disclosed further heterogeneity. For instance, 30 percent of black Americans have a deletion of one of the alpha-chain genes (- alpha/alpha alpha), and about 2 percent are homozygotes with deletion of two genes (- alpha/- alpha). The more severe forms of alpha-thalassemia-hemoglobin H disease (- -/-alpha) and hydrops fetalis (- -/- -) have not been found in blacks. Further heterogeneity in the alpha-thalassemia syndromes was revealed by the finding that in approximately one-third of the Mediterranean alpha-thalassemias, all the alpha genes are present.

Understanding the molecular pathology of the delta, beta-thalassemia syndromes and of hereditary persistence of fetal hemoglobin has progressed. Application of restriction endonuclease mapping in the delta, beta-thalassemias has disclosed deletions of the beta and delta loci that extend near to the beginning of the delta locus. In the African form of hereditary persistence of fetal hemoglobin, deletions have been detected that include the delta and beta genes and extend into the intergenic sequence between the delta and gamma genes. Deletions involving the beta, delta, and A-gamma genes and inversions affecting this globin genomic region have been identified among the delta, beta-thalassemia defects. In addition, forms of hereditary persistence of fetal hemoglobin with no identifiable deletions have been detected.

Striking progress has been made in the delineation of the beta-thalassemia syndromes. Abnormalities, the existence of which could not have been predicted in the early 1970's, have been detected. The following different types of molecular defects have been characterized to date:

- Complete or partial deletions of the beta-globin genes ranging in size from a few hundred nucleotides to many tens of thousands of nucleotide base pairs (bp) in chromosomal DNA

- Nonsense mutations as a consequence of single nucleotide base substitutions or one or two nucleotide base deletions, resulting in termination codons within the coding sequence of a gene

- Mutations that affect the processing of transcripts of genes, such as single nucleotide substitutions or short deletions of nucleotides at the junctions between coding sequences and intervening sequences, thus preventing normal processing and excision of intervening sequences from globin gene transcripts

- Single base substitutions within the body of intervening sequences leading to alternative processing of globin gene transcripts and synthesis of abnormal mRNA's that have decreased stability.

Prenatal Diagnosis and Prevention

With the use of globin biosynthetic methods, it was shown that prenatal diagnosis of beta-thalassemia is possible. Subsequently, technology was developed that allowed testing of blood samples obtained through puncture of the placenta or through direct blood drawing from a placental vessel during visualization with fetoscopy. Data have been accumulated on the diagnostic accuracy and safety of these procedures. Prenatal diagnosis of beta-thalassemia syndromes using globin biosynthesis performed on fetal blood is now offered on a service basis in this country and in several centers around the world.

After the introduction of recombinant DNA technology, direct approaches were developed for the diagnosis of thalassemia using DNA from amniotic cells, and prenatal diagnosis of beta-thalassemia syndromes was first achieved. Subsequently, population studies for restriction endonuclease polymorphisms were performed that disclosed the presence of polymorphic sites containing alleles at a linkage disequilibrium with the beta-thalassemia genes, and the feasibility of prenatal diagnosis of beta-thalassemias was demonstrated with the use of restriction endonuclease mapping of DNA from amniocytes.

Patient Care

Clinical studies have demonstrated the effectiveness of hypertransfusion programs in decreasing morbidity, decreasing the development of dysmorphic features, and improving the general quality of life of the patient with homozygous beta-thalassemia. Subsequent clinical investigations have shown that hypertransfusion programs do not in fact lead to significantly higher

iron burdens compared to traditional low transfusion programs (presumably because of decreased gastrointestinal absorption of food iron and suppressed erythropoiesis in the hypertransfused patient). During this decade, the hypertransfusion regimen in beta-thalassemia syndromes was introduced into clinical practice.

Research advances have been made with regard to improved transfusion therapy. "Supertransfusion" (maintaining even higher levels of hemoglobin) results in lower transfusion requirements when the steady state is achieved. Methods have been developed to separate young red blood cells ("neocytes") from donor blood, and evidence has been obtained that transfusions of neocytes may decrease the frequency of transfusion requirements and thus decrease, in the long term, the total iron burden in thalassemic patients.

A major advance in the care of patients with Cooley's anemia followed clinical investigations of iron chelation using deferoxamine. It was clearly shown that semicontinuous subcutaneous infusion of deferoxamine is highly effective in chelating iron and that net iron balance or even negative iron balance can be achieved by this therapeutic modality, which has now been introduced into medical practice.

Research has been focused on developing chelating agents that are either more potent than deferoxamine or that can be taken orally. The major disadvantage of deferoxamine, which is the need for parenteral administration, would be overcome. While no alternative drug has yet become available for practical applications, several promising potential drugs have been developed and are under investigation.

Improved methodologies for diagnosis of heterozygous beta-thalassemia were developed during the decade and subsequently used by industry to produce commercial tests that facilitate the more accurate diagnosis of beta-thalassemia trait. These tests have helped detect heterozygous individuals and facilitated their education about risks and disease prevention.

State of Knowledge in 1982

The precise molecular basis for many different forms of thalassemia has been established. These include: complete and partial gene deletions of different extents; base substitutions that cause nonsense mutations; and mutations in intervening sequences that either abolish normal mRNA processing signals or create new anomalous processing signals.

In almost 100 percent of cases, prenatal diagnosis of thalassemia is feasible by fetal blood sampling with analysis of

globin chain synthesis, and in 50 to 70 percent of cases by the safer procedure of amniocentesis with analysis of amniocyte DNA for the presence or absence of polymorphic restriction endonuclease sites linked to the thalassemic gene.

Therapy of thalassemia has been improved over the last decade with the use of semicontinuous subcutaneous infusions of deferoxamine to remove the accumulation of excess iron that results from multiple transfusions. The potential increased efficacy of supertransfusion programs coupled with the use of age-selected red cells ("neocytes") is being investigated. Finally, screening tests have been improved for the easier detection (and subsequent counseling) of heterozygotes.

Program Goals 1982 to 1987

- Delineate the molecular pathology of thalassemia syndromes by the analysis of the fine structure and in vitro expression of cloned thalassemic globin genes as well as by studies of globin mRNA metabolism in intact thalassemic erythroid cells.
- Establish referral laboratories that offer molecular prenatal diagnosis on a service basis.
- Develop methodologies that allow simple and accurate estimates of iron overload.
- Develop an effective oral chelating agent.
- Explore the cure of thalassemia through bone marrow transplantation.
- Support research on gene transfer into hematopoietic cells.
- Support research on the cellular and molecular regulation of fetal hemoglobin synthesis.
- Support research on methods for improving transfusion therapy.

Research Activities 1982 to 1987

- Research on the molecular biology of thalassemia should be supported with high priority. The fine structure of thalassemia genes should be determined so that specific structural gene defects can be related to specific functional abnormalities. Recombinant DNA allows the

isolation of thalassemic genes, and rapid nucleotide-sequencing technologies can provide a great deal of information about nucleotide sequence on these genes, such as identification of intervening and flanking DNA sequences and abnormalities that may be responsible for altered or defective expression. Emphasis should be given to studies of mRNA synthesis and metabolism in those forms of thalassemia where the globin genes are present and functioning at a lower than normal level. In beta^{+}-thalassemia and in nondeletion forms of beta-thalassemia, the defective globin genes are present and express a lower than normal level of their respective mRNAs. Current evidence suggests that several thalassemia syndromes are due to DNA structural abnormalities that affect mRNA processing. It is expected that studies of the thalassemia mutants will provide a large amount of information about abnormalities that affect mRNA processing and will clarify the normal mechanisms by which the precursor mRNA attains its mature form. Theoretically, in addition to the defects in processing, structural abnormalities are predictable when the transcription of mRNA from the genes is diminished or when the transcription is normal but metabolism and transport of mRNA molecules are defective. Studies of globin gene transcription in intact cells and in cell-free systems should shed light in this area. Improved techniques are required for the study of pulse-labeled mRNA from human bone marrow cells or erythroid cells grown in short-term culture systems.

The establishment of transformed permanent erythroid cell lines from thalassemic patients would also provide a useful system for the study of thalassemic globin gene expression. The availability of cloned thalassemic globin genes and the possibility of introducing them into SV40 or other animal viral vectors provide the ability to study the expression of these defective globin genes in an in vitro system. Further characterization and application of such systems to the study of thalassemic genes should be encouraged. Finally, the refinement of cell-free transcription systems and their application to the study of transcription of defective thalassemic genes obtained through recombinant DNA techniques should be encouraged.

- The semicontinuous subcutaneous iron chelation treatment is cumbersome and expensive. Emphasis should be given to the development of a chelating agent with either a simpler route of administration (such as oral) or a longer in vivo half-life. The development of new chelating agents falls into the realm of the pharmaceutical industry, especially since the apparent financial success of deferoxamine provides a profit motive. In the absence of interest by

the private sector, research to improve iron chelation should be supported.

- The therapeutic usefulness of bone marrow transplantation in patients with beta-thalassemia should be explored. Although the posttransplantation mortality may initially discourage many parents from accepting this form of treatment for their children, attitudes may change if a high therapeutic efficacy of the procedure is obtained, as is the case in aplastic anemias of childhood.

- Since prenatal diagnosis can be performed only in a few research laboratories, prenatal diagnosis using endonuclease mapping cannot, in practical terms, be offered today by practicing physicians. Establishment of laboratories that can provide standards of reference is recommended. It will be a great disappointment if the advances of molecular biology can not be made readily available to the at-risk population.

- Since young red blood cells live longer than older cells and since young cells can now be separated from older ones, there are possibilities for improving transfusion therapy for thalassemia.

- Simplified methods to detect carriers of the beta- and alpha-thalassemia syndromes should be encouraged. Simple and accurate methods for immunochemical measurement of relevant hemoglobin fractions should be developed for the diagnosis of beta-thalassemia carriers.

- Since premarital screening in combination with genetic counseling has not been shown to be very effective for disease prevention, other types of prevention programs should be encouraged. Postmarital screening with the option of prenatal diagnosis, for example, should be investigated, provided that these services can be offered by practicing physicians through reference laboratories with capabilities for prenatal diagnosis. A limited number of demonstration programs should be initiated to test the effectiveness of any new prevention strategy.

- As a possible future form of gene therapy for thalassemia, studies of gene transfer into hematopoietic cells should be encouraged. This technique would require additional knowledge about the maintenance and proliferation of erythroid stem-cells in pure culture to serve as host cells for gene transfer experiments. Also in this regard, the development of animal model systems for globin gene transfer experiments should be encouraged.

- The stimulation of production of erythroid cells with efficient synthesis of fetal hemoglobin may have therapeutic effects on beta-thalassemia syndromes. Research on the molecular regulation of fetal hemoglobin synthesis and on the control of hemoglobin switching in hematopoietic cells in vitro as well as experimentation with animal models should be encouraged.

5. Blood Resources

Contents

5. Blood Resources

The National Program of 1972 focused on several aspects of the blood resources program of the Division of Blood Diseases and Resources. Its primary goals were:

- Achieve an adequate supply of high quality blood and blood components* by utilizing the national blood resource with maximal efficiency, economy, and safety.

This effort was viewed in the program as directly dependent on the creation of a regionalized, nationwide system supplied by volunteer donors. The collection of data on usage was considered to be a major consideration in the accomplishment of this goal.

- Assure the safety of blood and blood products.

While posttransfusion hepatitis due to hepatitis B virus was the major concern of the program, the emerging importance of cytomegalovirus highlighted the 1972 report. The need for more information about histocompatibility antigens was mentioned, as was the necessity for avoiding human errors by developing fail-safe systems in transfusion therapy.

- Reduce the use of whole blood in favor of component therapy.

The need for research on better methods of separation, storage, and preservation of components was stressed. An educational

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*In the common language of blood banking, blood "components" usually means the red cells, white cells (or particular subsets thereof, such as granulocytes), platelets, cryoprecipitate, and plasma in its many forms (frozen, liquid, platelet-poor, cryoprecipitate-poor). Blood "derivatives" are considered to be products that have undergone steps of manufacture--such as protein fractionation, sterilization, and freeze-drying--beyond those generally performed in blood banks.

A simple organization has been followed in this section of the report. Cellular components are considered individually, whereas plasma and all therapeutic materials prepared from plasma are discussed under a single heading.

program focusing on the indications for and optimal use of components was felt to be crucial.

- Improve the handling, preservation, and use of human materials suitable for transplantation.

In addition, a need to develop special centers of research in blood resources was discussed in the program. It was believed that in such an environment, scientific advances could be more effectively applied to increase the quality and number of blood products, and efforts could be coordinated for addressing a variety of clinical and technical problems.

The 1976 Report of the Blood Resources Ad Hoc Work Group to the Director, DBDR, not only expanded these considerations and provided an extensive review of progress at that time, but also suggested new directions for the Division to pursue in the coming years. Additional information has appeared in annual reports of the Director, National Heart, Lung, and Blood Institute. These data are presented below in discussions of particular topics.

Therapeutic Uses of Blood and Blood Components

The value of blood transfusions has long been recognized as a means to restore a volume of circulating blood lost by hemorrhage, and it has become a practical routine for coping successfully with blood lost through accidents or in surgery. Discoveries of the biological properties of blood constituents during the past decade have greatly expanded the health benefits derived from the use of blood components in increasingly specialized and effective therapy. Progress has also been made as a result of the development of a new kind of health care facility, the regional blood center, which organizes public participation in donating blood, houses specialized equipment and personnel for testing and separating blood constituents, and centralizes management and distribution of blood and blood components. Techniques have been adopted that eliminate donations having detectable transmissable agents of disease; advances in the separation of blood into constituents have enabled specific therapies to be introduced; and discoveries of new constituents of blood have broadened the range of therapy being utilized. These advances have changed transfusion practices of hospitals, improved safety in transfusions, made blood resource management more efficient, and increased the availability of blood in patient care.

The clinical importance of advances in transfusion therapy is best understood in the context of the large numbers of recipients of transfusions. The most striking figures revealing this dependence are that 5 percent of all hospitalized patients require blood

transfusion and that, although only 22 percent of these patients are over the age of 65 years, 44 percent of blood transfusions are administered to the older group. Cancer and heart disease, the two major health problems in the United States, are the leading diseases for which transfusion is required. In fact, modern therapy for both would be impossible without an effective transfusion system.

Although the percentage of whole blood units transfused without separation into components has declined dramatically over the past decade, recent data show that a significant number of whole blood units are still transfused. According to a study supported by the NHLBI, 4,972,400 units of whole blood were transfused in 1971. This number represented 56.6 percent of the units collected by hospitals and blood banks. According to statistics provided by the National Blood Data Center collected from 6,751 hospitals and community blood centers throughout the country, 2,165,321 units of whole blood were transfused in 1979, representing 19.5 percent of the units collected.

The numbers of cellular components that are transfused have increased dramatically over the past decade. According to the NHLBI study, 1,394,100 units of red cells were transfused in 1971. According to statistics provided by the the National Blood Data Center, 7,306,449 units of red cells were transfused in 1979, representing a 424 percent increase for the period. Similarly, 413,500 units of platelets were transfused in 1971 and 2,219,573 units in 1979, for a 437 percent increase in platelet utilization.

Transfusions of red blood cell products increased 48.8 percent from 1971 to 1979. During this period, the U.S. population increased only about 11 percent. (The percent of the population having access to modern medical therapy undoubtedly increased during this interval, but quantitative estimates of this change are not available.) While there are no specific analytical studies of increase in blood usage during the past decade, advances in certain medical and surgical practices are likely responsible. Aggressive, early treatment of victims of severe trauma and burns, for instance, relies heavily on transfusions of blood products. The frequency of complex surgical procedures that require multiple transfusions, such as open heart surgery, has increased dramatically. The current intensive therapy of acute leukemia requires transfusion of multiple blood components, particularly platelets and to a lesser extent granulocytes. Adequate cellular replacement therapy of leukemics directly correlates with improved survivals.

Very little information exists on the utilization of plasma and plasma derivatives in the United States. Statistics from the National Blood Data Center provide information on the production

and utilization of whole blood, red blood cells, and other cellular components. Data on the utilization of plasma derivatives, however, are not included. The only relevant information that can be gleaned from 1979 census statistics combines total amounts of plasma and cryoprecipitated antihemophilic factor transfused. In 1979, 1,285,686 units of plasma were administered. This number represents a 601 percent increase in plasma utilization since 1971, when 183,300 units were administered. In 1979, 412,586 units of cryoprecipitated antihemophilic factor were administered. This number represents a 1.9 percent increase since 1971, when 405,000 units were administered.

The American Blood Resources Association, which is an organization of most of the commercial plasma collectors, releases information on the production of plasma and plasma derivatives. According to its statistics, 4.2 million liters of plasma obtained by plasmapheresis and intended only for fractionation ("source plasma") were produced in 1979; 80 percent of this plasma was used for fractionation in the United States.

According to these same statistics, 72.5 million grams of albumin and 412.5 million units of factor VIII, supplied as antihemophilic factor preparations, were used in the United States in 1979. It is not known whether these data represent a comprehensive analysis of the industry or were obtained by sampling.

Progress and State of Knowledge Through 1982

Advances, which are discussed later in detail, are summarized below.

Management of Blood Resources

For blood to be therapeutically effective, it must be readily available at the hospital where the patient needs it, but because of the complexity of effective blood inventory management, this obvious requirement has presented serious and challenging problems. The situation is due to the large number of combinations of blood groups and types that must be on hand, the limited shelf life of such living tissue, its relative scarcity, and an inability to know with certainty where sudden needs will arise.

The National Blood Policy* and the Regionalization Program of the American Blood Commission, which was organized in response

*Issued in 1973 by the Secretary of Health, Education, and Welfare.

to the Policy, have enunciated the importance of regionalization in improving inventory management. As a result, regional blood centers have been organized as focal points for the acquisition and distribution of blood to area hospitals. The centers have made a major contribution to the solution of this problem.

Inventory management requires innovative strategies for coordinating the use of blood within a region in a way that affords equal protection for all patients. This problem of management can be, and has been, effectively resolved with the development of area-wide computer-supported policies and procedures for the distribution and redistribution of inventories. Housed at a regional blood center, a centralized system of information analyzes patterns of hospital usage and predicts inventories of blood that would be required for meeting both common and rare needs. Since the amount needed for rare events is high and since maintaining such levels can predispose to outdating, redistribution of blood from hospital to hospital by the regional center becomes an essential operation. Fresh blood delivered to small, low-usage hospitals can be kept "on shelf" in case of need, and can be redistributed, with an acceptable storage life, to large, high-usage hospitals. This approach, which requires sophisticated mathematical models and computer programs, has transformed the management of hospital blood banking from "crisis" responses to skilled, planned inventory control.

Two important advances in preserving cells have occurred during the past 10 years, with direct implications for all blood centers, blood banks, and patients. The first is an extension of shelf life for red cells. The Bureau of Biologics has recognized that adenine can improve the ex vivo storage time of red cells and has licensed CPDA-1 solution. CPDA-1 solution extends the period of storage from 21 to 35 days. Ongoing research has led to a new solution that extends shelf-life to 42 days, and its licensure is anticipated shortly.

The second advance is in the use of platelets. Platelets are essential for the control of bleeding, and they have been increasingly used in the treatment of patients with cancer and leukemia. Fully one-third of all blood collected in some regions is now used by the regional blood center to prepare this component.

Platelets are fragile and are subject to rapid deterioration during storage, with subsequent loss of therapeutic potency. During the past 10 years, optimal storage conditions have been defined, and shelf-life has been extended from 1 day to 5 days. These developments have led to more rational management of the resource, enhanced availability, decreased wastage, and improved patient care. The improvement in patient care is exemplified by the fact that bleeding, once the major cause of death of patients with leukemia, in most instances is no longer a major threat.

Autotransfusion

Autotransfusion is the collection and reinfusion of a patient's own blood. While this technique has been an acknowledged method for over 150 years of providing blood for patients with massive bleeding, it is only in the last 5 years that the potential for autotransfusion has been examined. In the early 1970's, cumbersome equipment and poor safety measures caused a number of incidents that led almost to the extinction of the use of autotransfusion. A number of technical obstacles, however, have been overcome, and safer devices have been marketed. As a result, renewed interest in autotransfusion occurred during the mid-1970's.

Transfusing patients with their own blood has certain advantages over homologous blood. Hepatitis, for instance, is still a major problem of transfused homologous blood. Autotransfused blood is free of hepatitis virus unless the patient already has hepatitis. The use of autologous blood can reduce the incidence of posttransfusion hepatitis, the number of deaths associated with it, and the cost to the health care system of treating patients who have it. Other risks associated with transfusion of homologous banked blood include isosensitization, febrile and hemolytic transfusion reactions, and technical error. These problems can be eliminated when a patient receives his own blood. Increased use of autologous blood can also increase the availability of blood in a given community during times when the number of donations is low and the demand is high, such as on holiday weekends.

There are currently five methods of autotransfusion: predeposit, in which prior to surgery a patient donates blood, which is stored as whole blood or frozen, depending on the time involved; perioperative phlebotomy with concomitant hemodilution, in which whole blood is drawn from a patient after anesthetic induction and replaced with colloid or crystalloid; intraoperative salvage of shed blood; collection and reinfusion of blood from a traumatic hemothorax in the emergency room; and postoperative salvage of shed mediastinal or thoracic blood. Because of defibrinogenation, mediastinal and chest blood does not clot, but normal levels of most other clotting factors are noted. The mechanism by which prothrombin is activated in shed blood is currently being investigated. Understanding this mechanism might open new avenues for a better understanding of the interactions between blood and tissue surfaces.

Studies have indicated that patients who receive their own blood have fewer postoperative infections than patients receiving homologous banked blood. This finding suggests that the process of blood collection and reinfusion activates some aspect of the immune system. This concept requires careful investigation. If such is the case, reinfusion in patients requiring orthopedic

surgery, where infection is always a potential problem, may be especially valuable; and in certain kinds of cancer where surgery is required, reinfusion might enhance the ability of the immune system to seek out and destroy tumor cells. Such studies of immune function of shed blood are needed.

The potential impact of autotransfusion on the availability and cost of blood to the patient could be significant. It has been estimated that between 5 and 8 percent of the transfusion requirements of this country can be met with autologous blood, but no studies have as yet been made that support this projection. Efforts to gain a better understanding of the potential influence of autotransfusion on blood resource management in the United States should also be made.

Prevention of Transfusion Reactions

Transfusion reactions occur with a frequency of 1 to 2 percent. Fortunately, most of them are mild and do not seriously compromise the recipient, but some can be serious and occasionally fatal. Acute reaction with rapid destruction of red cells is usually due to mismatching of blood type and may result in serious disability or death. It is only rarely due to technical error; it is almost always due to an error in clerical procedures related to transfusion--such as confusion of specimens, improper labeling, or transfusion of the wrong unit. The frequency of clerical errors has been shown in the past to be as high as 1 per 1,000 transactions. The high rate of clerical error and the severity of the transfusion reaction that it caused stimulated research into methods to decrease the frequency of error by automation of techniques of blood grouping and by computer-controlled record processing and record keeping in laboratories. These efforts have yielded methods of control that are almost free of error. The rate of error has been reduced to 1 or less than 1 per 1 million transactions.

Blood centers have thus far been the primary beneficiaries of these advances, but in the next 10 years these technologies should be extended to hospital settings as well. The use of specifically designed laser-readable bar-code labels of packaged blood units matched with labels for patients (now familiar markings on supermarket merchandise) have been the basis for this progress in technology.

Acute hemolysis can also be caused by antibodies against red cell antigens other than the blood-type ABO antigens. Since such reactions can be associated with serious sequelae, they present a challenge to the blood bank. The need to identify and characterize such antibodies is demanding and can often strain the personnel and technical resources of an individual blood bank. As more patients require transfusions, more persons enter the pool of

recipients with antibodies. The development of methods of computer-controlled analysis and interpretation of the complex data needed for solving antibody problems is under way. Staff with expertise to handle these complex technical problems is limited, and advances in automation should improve the ability of hospitals, small and large, to handle antibody problems and prevent transfusion reactions.

Another category of reaction, repeated high fevers, is seen in frequently transfused patients. These reactions have been shown to be due to antibodies in the patient to leukocytes in the transfused material. Prevention is best achieved by removal of the leukocytes, and removal is best accomplished by use of frozen red blood cells.

Prevention of Maternal Sensitization

In the past 10 years, erythroblastosis fetalis due to Rh incompatability between mother and fetus has been almost entirely eradicated. The critical observations upon which this historic achievement was based were made during the 1960's. It was shown that Rh positive red cells, coated with antibody to the Rh factor, did not produce sensitization in recipients of these red cells. The importance of this observation to the Rh negative woman carrying an Rh positive infant became apparent when it was shown that administration of antibody to the Rh antigen at the time of first delivery of an Rh positive child prevents development of her own antibody response. This procedure permits her to have subsequent children without the complications of neonatal anemia, jaundice, heart failure, and, frequently, death.

Prevention of Bleeding in Hemophilia

In 1964, Dr. Judith Pool made the seminal observation that factor VIII can be isolated by cryoprecipitation from large volumes of plasma. Following this observation, better concentrates of factor VIII were manufactured, which permitted outpatient care of hemophiliacs, then home care, and then self-administered prophylaxis. Hemophiliacs can now lead normal lives, attend school, and take jobs. This advance has changed the life of the hemophiliac from one marked by suffering and disability to one in which the affected person can enter the mainstream of society.

About 10 percent of patients with hemophilia develop an inhibitor to factor VIII that interferes with conventional therapy and can be life-threatening. A newly developed plasma product that contains activated clotting factors can circumvent the block caused by the inhibitor. This development has brightened the

outlook for this group of hemophiliacs and should yield improved management strategies.

Prevention of Hepatitis

Posttransfusion hepatitis, long recognized as a serious risk of blood and blood component therapy, was significantly brought under control during the 1970's as a result of the demonstration that the hepatitis B virus, which causes a substantial proportion (approximately 30 percent) of the cases of posttransfusion hepatitis, is detectable in the plasma of donors. Eliminating the use of blood from positive donors has resulted in an almost total elimination of hepatitis B posttransfusion hepatitis. In addition, hepatitis B immune globulin (HBIG), obtained from people who have previously had hepatitis B infection and become immune to it, has been shown to be effective in preventing hepatitis B infections in individuals accidentally exposed to the virus (such as by needle stick) and in newborn infants of mothers who carry the virus. More recently, a vaccine prepared against hepatitis B has been successfully tested for both clinical safety and efficacy, and it was licensed in 1981 by the Food and Drug Administration. Several million persons at high risk of hepatitis B infection in the United States can soon look forward to protection from this serious illness. In addition, the potential for improving worldwide health cannot be overstated, since hundreds of millions of the world's populations suffer from infection by this virus, and some succumb to a late complication of the illness, cancer of the liver.

Soon after the introduction of hepatitis B testing, cases of posttransfusion hepatitis unrelated to hepatitis B testing were observed. An NHLBI-supported, major epidemiologic study demonstrated that non-A,non-B hepatitis follows almost 10 percent of all blood transfusions and that the liver enzyme alanine aminotransferase (ALT), which is present in small amounts in the plasma of all individuals but in increased amounts in persons with liver injury, may be an indicator of high-risk donors. Further examination of this relationship is under way.

Prevention of Cytomegalovirus

It has been demonstrated that cytomegalovirus, which is ubiquitous in the population, can be the source of fatal infections in premature infants given transfusions. Clarification of this association has led to control of this infection in such infants by the introduction of sophisticated procedures to select donors and by innovative strategies of inventory management to provide for this vulnerable population specially tested blood that is free of CMV.

Prevention of Allograft Rejections

A startling and unexpected finding in the field of blood transfusion therapy came to light recently in studies of kidney graft rejection. Accumulated data now clearly indicate that blood transfusions prior to kidney transplant decrease the likelihood of rejection of the kidney. Although the immunologic basis for this finding has yet to be defined, the clinical implications have been rapidly appreciated and quickly put into widespread use.

Blood Components in Cancer and Heart Disease Therapy

Chemotherapy frequently results in life-threatening leukopenia, severely compromised immune competence, thrombocytopenia, and hemorrhage. Administration of platelet or leukocyte concentrates has become a routine practice during the past decade and is an essential adjunct to cancer therapy. Progress has also been made in supporting patients with crippled immune responses by the administration of immune globulins, granulocyte concentrates, and interferon.

Potentially lethal doses of radiation can now be administered to eradicate malignant disease and can be followed by restitution of the patient's bone marrow with compatible marrow and supportive hemotherapy during the recovery phase. Further developments in immune tolerance and other approaches to immune modulation hold promise for long-term survival among persons receiving such therapy.

Widespread use of coronary bypass surgery for the treatment of coronary artery disease draws heavily on the supply of blood for transfusions. It is estimated that 10 percent of the total blood supply is now used for open heart surgery.

The Use of Other Specific Immune Globulins in Therapy

The common childhood disease, chicken pox, which usually causes discomfort and mild illness in normal children, can be devastating in an immunosuppressed child with leukemia or cancer, and in adults. New methods have been developed for removing varicella immune globulin from plasma, and they permit its manufacture and widespread availability for preventive therapy. Indications for this product have been defined, and its distribution has been made possible through regional blood centers throughout the United States.

Rabies immune globulin has also become available for administration in conjunction with rabies vaccine in those instances in which exposure to this virus has occurred.

Apheresis

It has recently been recognized in patients that failure of response to platelet transfusion can be the result of platelet destruction. The histocompatible antigen (HLA) system has been found to be important in preventing such reactions. Platelets of HLA-matched donors offer the best therapeutic hope for sensitized recipients, and since large numbers of matched donors are difficult to locate, apheresis techniques have been adapted to help solve the problem. In this procedure, an extracorporeal circuit is established, a donor's blood is centrifuged, selected cells are harvested, and the remainder of the blood is returned to the donor. Application of this procedure permits the collection of a therapeutically adequate amount of platelets or leukocytes from one HLA-matched donor. Files of such donors are maintained at blood centers throughout the United States, and a new way to treat people who are seriously ill has been developed.

Therapeutic apheresis, which is the selective removal of certain cellular elements or plasma constituents from the blood of a patient, has become a widely used therapeutic modality. It is encouragingly successful in some diseases, and still experimental in others and under further study.

Restitution of plasma volume during therapeutic plasmapheresis relies on infusion of corresponding volumes of human albumin solutions. Because these solutions do not presently include balanced electrolyte formulations, they are not entirely satisfactory. Progress has been made in the selective removal of offending constituents from patients being treated by plasmapheresis. Blood cells are separated extracorporeally in a sedimentation field. The supernatant plasma flows through matrices that are capable of binding the pathogenic constituent or selectively filtering it, or the plasma can be passed through low-temperature zones that are capable of precipitating and separating immune complexes. The patient's extracted plasma is then returned with the blood cells to the circulation. Specialized teams comprised of experts in a specified disorder, blood separations technology, and fluid-balance physiology must be formed for properly conducting these procedures.

Needs and Opportunities 1982 to 1987

Areas for future research, which are discussed later in detail, are summarized below.

Membrane Engineering

The conversion of group B to group O blood creates an opportunity to extend this seminal event to other blood groups and other cells. The body recognizes certain cell surface substances as foreign. The ability to remove such substances selectively provides a base for advances in the entire field of transplantation biology.

Lymphokines and Other Mediators

Interferon, which is one of a series of molecules secreted by cells of the immune system, has potential therapeutic importance to patients with viral, malignant, and immune system disorders. Many other lymphokines have been identified but not yet characterized or fully explored. New techniques for isolating and culturing cells and for identifying and characterizing mediators will lead to rapid growth in the understanding of these molecules, their interactions, and their therapeutic potential.

Plasma Proteins

Of the more than 100 proteins known to be present in plasma, only 10 percent have been sufficiently characterized or isolated for clinical use. Increases in the knowledge of the function of many of these proteins will present major opportunities in the field of blood therapy. Uses of fibronectin and antithrombin III, for instance, show promise. In addition, a new approach to the production of gamma globulin has resulted in preparations for intravenous use. Further progress in the development of clinically useful, additional plasma products will undoubtedly be facilitated as a result of the availability of improved methods for controlling the hepatitis B virus.

Blood Substitutes

Human blood is a complex mixture of cells, plasma, proteins, and small molecules. Each of these serves a specialized function(s). Individual substitutes capable of meeting such functions are being developed. Fluorinated hydrocarbons have already been shown, under selected circumstances, to have potential for the oxygen-carrying function of the red cell. Other red cell substitutes, such as stroma-free hemoglobin, are under investigation. Plasma volume expanders that replace albumin as a source of oncotic pressure, such as dextran and hydroxyethyl starch, have been developed and are in use. These substitutes, however, have serious shortcomings, and better agents need to be designed to combat shock.

Blood Collection and Separation

Presently available techniques for collecting blood and separating it into plasma and cellular components are laborious, lengthy, and expensive, and new methods are under development. Membrane plasmapheresis is one of them. It will permit the removal of large volumes of human plasma as conveniently and as quickly as a pint of blood is now collected, without the risks of conventional plasmapheresis. Methods need to be developed to further purify components, and technologies for doing so are emerging.

Autotransfusion

The use of autologous blood is considered to have a significant potential in selected situations for alleviating some of the strain on blood resources. To learn more about the physiologic and pathologic changes taking place in shed blood, basic research is needed.

Safety of Donors

The major source of the substances that make all transfusion therapy possible is the individual donor. Concern for the safety of the donor should be increased to ensure that excessive quantities of fluid or cells are not removed, that critical cell populations are not depleted, and that the donor's immune mechanism is not compromised. Long-range studies and surveillance programs of donors should be initiated.

Red Blood Cells

Red blood cells that are used as a blood component should be free of other blood cells and plasma and of any infectious or potentially toxic materials, such as leachable plasticizers. Theoretically, transfusions of such a "pure" product do not elicit in the recipient immunization to other blood components, do not lead to transfusion reactions due to preformed white blood cell antibodies, and do not transmit viral diseases or expose the recipient to potential carcinogens.

Red blood cells prepared by current techniques, however, contain other blood cells and plasma in variable amounts. In addition, the function and viability of red blood cells deteriorate during storage. Research in the area of blood preservation has identified new preservative solutions and techniques of storage that maintain red blood cell viability and function longer

than was previously possible. Some of these techniques, however, are time consuming and add to the cost of blood.

The specific goals set by the Institute in 1972 relating to red blood cell components were:

- Limit the use of whole blood to the cases requiring such treatment. It was estimated that 25 percent or less of the patient population requires whole blood transfusions.
- Support basic and applied research to improve methods for preservation of whole blood during storage and establish and disseminate clearer indications for the clinical use of red blood cells.
- Advance the technology and methodology of red cell immunohematology.

In 1976, the Blood Diseases and Resources Advisory Committee Report listed the following progress since 1972:

- Red blood cell storage had been increased to 35 days with the use of new additives.
- The importance of pH in relationship to red blood cell preservation had been established.
- The importance of 2,3-DPG had begun to be appreciated.
- A sterile docking device had been developed.
- The importance of microaggregates was being investigated.

The advisory committee, in turn, recommended that:

- Additional effort be made to increase red blood cell viability to 45 days while maintaining acceptable 2,3-DPG levels.
- Studies be made of frozen red blood cells.
- Efforts be continued on ways to free red blood cells of contaminating white blood cells and antigens.
- Work be undertaken to determine the significance of red blood cell antigens.
- Support be provided for the development of new quantitative antibody antigen reactions.
- Standardized blood bank reagents be developed.

- Clinical trials of the sterile docking device be undertaken.
- Industry develop better instrumentation for blood banks.
- Further evaluation of microaggregates be undertaken.

In 1977, the Fourth Report of the Director, NHLBI, noted progress in the long-term storage of frozen red blood cells containing rare blood groups, in the evaluation of a closed sterile blood system for the preparation of thawed red blood cells, and in the generation of data on optimal 2,3-DPG levels. A plea was made for additional training of the medical community on proper transfusion practices.

As indicated in the Fifth and Sixth Reports of the Director (1978 and 1979), the Food and Drug Administration licensed CPD-adenine as a preservative that would extend the shelf life of blood to 35 days. In the Eighth Report (1980), the development of a new preservative was announced that could sustain red blood cell storage for 6 weeks with maintenance of adequate 2,3-DPG levels. At the same time, decreased 2,3-DPG levels were noted to be of potential value in preventing sickling of sickle cell red cells in vivo.

The nation's blood resource was more efficiently utilized at the end of this period than at the beginning. About 21 percent of all red blood cell transfusions in 1971 were given as components, and the remainder as whole blood. In contrast, about 77 percent of all red blood cell transfusions in 1979 were given as components, and about 23 percent as whole blood. Because of the use of component therapy, a slight increase from 1971 to 1979 in the number of whole units collected served the needs of almost twice as many patients who required some form of transfusion therapy.

Progress and State of Knowledge Through 1982

Red blood cells and other blood components are prepared and stored in plastic bags made of polyvinylchloride (PVC), which contains the plasticizer di-2-ethyl-hexyl phthalate (DEHP). The DEHP is not bonded to the polymer matrix, but is interspersed between adjacent PVC chains where it acts as a lubricant. Therefore, DEHP can migrate out of the plastic and accumulate in donor blood during storage. The same phenomenon can take place during the circulation of a patient's blood through plastic equipment, as occurs during renal dialysis and open heart surgery. Concern has been expressed about the potential toxicity of DEHP to human beings since DEHP in large doses has been reported to be hepatotoxic and carcinogenic in rodents.

During storage of whole blood for 21 days at 4°C, about 1 mg DEHP per day leaches into a unit of whole blood. During the same period, about one-half this amount of DEHP accumulates in a unit of packed red blood cells. Platelet concentrates accumulate DEHP at a more rapid rate, and after storage at 22°C for 3 days, a unit contains from 7.2 to 15.2 mg DEHP. Over 95 percent of the DEHP resides in plasma where part of it is metabolized to mono-2-ethylhexyl phthalate (MEHP) under the influence of nonspecific lipases. The rate of this hydrolysis is dependent on the kind of PVC used to manufacture the bag. Washing red blood cells removes over 95 percent of the DEHP from the transfusable product. DEHP is rapidly eliminated from the body after transfusion, with about 98 percent being excreted in urine.

At present, it is not clear whether phthalate esters pose a health hazard to transfused patients. It should be noted that DEHP is ubiquitous in today's "plastic" environment and that DEHP has been identified as a pollutant of water and air. Although newer plastics have been developed free of leachables, they unfortunately do not maintain acceptable red cell viability during storage.

Liquid Preservation

All stored red cells should ideally have normal post-transfusion viability (that is, all transfused red cells should be recovered in the recipient's blood stream at 24 hours) and function (that is, provide delivery of adequate oxygen to tissues). During storage of blood and packed red cells at 4°C, however, red blood cell function and viability deteriorate. Although many changes occur during liquid storage, it is not known which metabolic lesion(s) is directly responsible for the loss of viability. It is suspected that the gradual decline in the adenosine-triphosphate concentration, which is the major source of energy for red cells, is an important factor. A statistically significant negative correlation has been established between ATP concentration and the percent viability of stored red cells. Secondary to the decrease in levels of red blood cell energy (ATP) during storage, levels of intracellular potassium decrease and levels of sodium increase. Other alterations include intracellular vesicle formation and loss of membrane sialic acid, decreased deformability, and increased osmotic fragility. The membrane changes resulting from storage have all been described as individual defects in clinical disorders known to be associated with increased rates of red cell destruction. It is considered possible, therefore, that membrane alterations during storage might make red cells nonviable. Immunologic phenomena have also been reported, such as an increase in membrane-bound C3 (the third component of human complement) and IgG. The influence of these immune phenomena on the viability of stored red cells has not yet been evaluated.

The decrease in delivery of oxygen of stored red cells is caused by a decline of their 2,3-diphosphoglycerate concentration. Loss of 2,3-DPG is accelerated when red cells are stored in an acid medium. Such a medium promotes a rapid drop in intracellular pH.

The goal of recent work on blood preservation has been to maintain almost normal concentrations of the biochemical metabolites that control function (as indicated by 2,3-DPG activity) and possibly viability (as indicated by ATP concentration). First, an acid-citrate-dextrose (ACD) anticoagulant preservative solution was developed. Red blood cells stored in this solution maintain acceptable viability for 21 days. It was later recognized, however, that the function of ACD-stored red blood cells was drastically impaired after 5 days because of the decline of 2,3-DPG due to the acidity of the anticoagulant. The development of citrate-phosphate-dextrose (CPD) solutions has allowed function to be maintained far longer than was possible with ACD, with comparable survival times in vivo. The maximum storage period using CPD anticoagulant, however, was still restricted to 21 days.

In order to improve inventory control of red blood cells, the objective of the last decade was to extend the time of storage and also maintain an acceptable level of function and viability. It has been possible to maintain the ATP concentration of stored red blood cells for at least 35 days by supplementing the preservative solution with additional glucose and adenine, which is the purine source for ATP. The maintenance of acceptable viability at the end of 35 days of storage, however, has not been unanimously documented by all investigators. Other formulas in which the concentration of adenine is doubled and more glucose is added (CPDA-2 and CPDA-3) are presently being tested in an attempt to lenghten the period of storage to 42 days.

Although supplementation with adenine improves levels of ATP, this preservative adversely affects 2,3-DPG concentrations. Such concentrations remain optimal for only 14 days. To maintain 2,3-DPG, additional preservatives are being evaluated. Vitamin C, dihydroxyacetane, and methylene blue may stabilize 2,3-DPG levels in red cells supplemented with adenine by facilitating the oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD).

Because of unknown effects of these additives on other blood components, initial separation of components prior to adding preservatives is being explored. The additives adenine and glucose are supplied in a solution of 0.9 percent sodium chloride (SAG solution). During the 5-week storage period, red cell breakdown was reduced by removing residual white cells, and the hemolysis could be further decreased by supplying mannitol. The viability

of red blood cells stored in this solution was maintained for 5 weeks, but their function was impaired after only 2 weeks.

Despite these attempts to improve inventory control by extending the shelf-life of blood, about 7.5 percent of red blood cells become outdated nationwide. An approach to this problem has been the use of rejuvenation solutions. Both the viability and function of metabolically depleted red blood cells can be restored by such solutions. The process involves incubating packed red blood cells in a solution containing adenine (for regeneration of ATP) and phosphate, inosine, and pyruvate (for regeneration of 2,3-DPG). Cryopreservation of these rejuvenated red cells with glycerol can extend storage time indefinitely. Compared to fresh red cells, such cells have a 91 percent in vitro recovery and 82 percent posttransfusion survival. The 2,3-DPG concentration of outdated but rejuvenated red blood cells can be restored to values between 100 percent and 150 percent of normal. If the rejuvenation process is applied early in storage, the concentrates can be increased to between 200 percent and 300 percent of normal. Hazards of the process are potential bacterial contamination either at the incubation stage with the rejuvenation solution or during the subsequent washing, which is essential for removing the rejuvenation solution before transfusion. Inexpensive sterile docking devices are needed to facilitate transfers of solution without contamination.

Frozen Preservation

While being frozen, red blood cells must be protected against injury that is caused by the formation of ice crystals. Although rapid freezing reduces the number and size of the crystals, either extracellular or intracellular additives must be employed to bind free water and prevent crystallization. If extracellular additives are used in red blood cell freezing, it is theoretically unnecessary to remove them prior to transfusion. Two macromolecules, polyvinylpyrrolidone (PVP) and hydroxyethyl starch (HES), which cannot penetrate the cell membrane, have been used in this fashion. When these additives were employed to freeze red blood cells at the temperatures of liquid nitrogen, however, an unacceptable degree of hemolysis was produced. In addition, there was some concern about possible toxic effects of infusing these additives. For these reasons, freezing techniques now use additives that move intracellularly, and they must be removed from thawed red blood cells prior to transfusion. Although various types of "antifreeze" solutions have been employed (dimethylsulfoxide, ethylene glycol, ethanol, methanol, trimethylammonium acetate, ammonium acetate, and glycerol), only glycerol has been extensively used clinically.

At the beginning of this decade, several methods of freezing had already been introduced into clinical practice. They used both high (about 48 percent w/v) and low (about 20 percent w/v) concentrations of glycerol. The high concentration method employed a slow rate of freezing, with storage at -80°C, whereas the low concentration method required a rapid rate of freezing, with storage at about -150°C (liquid nitrogen). Methods of storing and deglycerolizing the preparations were gradually developed.

A major technological improvement during the last decade was the development of a simplified process of freezing that uses the container in which blood is normally collected instead of a special, expensive cryogenic bag. In this method, the supernatant glycerol solution is removed by centrifugation prior to storage at -80°C. The method permits more efficient use of freezing space and a simplified process of deglycerolization. Frozen red blood cells of rare antigenic specificity are stockpiled for autologous transfusion programs and during periods of overcollection. Furthermore, since washed deglycerolized red blood cells contain only trace amounts of other blood cells and plasma, they approximate the ideal product.

Unfortunately, clinical experience with frozen blood has not measured up to expectations. Transmission of hepatitis has been observed in patients receiving deglycerolized red blood cells. Definitive studies have showed that chimpanzees become ill with hepatitis after receiving frozen red blood cells prepared from hepatitis-positive blood. In contrast, available data indicate that previously frozen, deglycerolized red blood cells do not immunize the recipient to other blood cells. This property, however, appears to be undesirable in certain clinical situations. Transfusion of whole blood and packed red blood cells rather than of deglycerolized red blood cells, for example, enhances the survival of kidney grafts. It has been postulated that transfusions elicit the development of "blocking" antibodies that protect the graft against cytotoxic antibodies and the actions of "killer cells."

These findings combined with the high cost of preparing frozen blood have caused a decline in the use of this product. Proper use of frozen red blood cells in transfusion practice remains to be determined.

Compatibility Testing

Pretransfusion tests must be performed to select donors with red blood cells that are compatible with a patient's serum. For several decades, the indirect antiglobulin test (AGT) has been used for this purpose. While most blood bank laboratories

continue to type blood and perform cross-matches by manual methods, many larger blood centers use automated techniques, and they also screen serum for irregular antibodies. Automated methods remove subjective variation and error in testing, permit more rapid testing, increase the number of tests that can be made in a given period, consume less reagents, and permit accurate computerized data analysis and storage.

Where antibody concentrations in the patient's serum are so low or the avidity of the antigen-antibody reaction is so weak that existing incompatibilities are not detected by the indirect AGT, immediate or delayed transfusion reactions can result. This is a significant problem with present methods. A retrospective study showed that a standard indirect test used to assess compatibility resulted in one delayed hemolytic transfusion reaction for every 11,652 units given, with a mortality rate of 13 percent. The incidence of subclinical delayed hemolytic transfusion reactions is probably higher than this percentage. It is recognized that more sensitive methods to perform pretransfusion cross-match tests are needed.

During the last decade, it was determined that incubation of donor red blood cells and patient's serum under conditions of reduced ionic strength improves the speed, sensitivity, and efficiency of detection of antibody. The exact ionic strength is critical for accurate detection. A slight decrease (to about 80 percent of normal) does not produce nonspecific complement uptake by red blood cells, and thus, polyspecific antiglobulin reagents can be used. However, if very low ionic strength conditions (about 20 percent of normal) are used, nonspecific uptake of complement components by red blood cells occurs. Monospecific anti-IgG reagents are required to maintain specificity of the reaction. Use of these low ionic strength tests, which have been adopted by many blood banks, has improved the effectiveness of detection of antibody. There has been a concomitant increase, however, in the incidence of nonspecific reactions.

Other types of methods to detect antibody have been evaluated. They use decreased ionic strength combined with selected additives, such as polybrene and protamine. These tests have not yet been universally adopted.

Not all blood group alloantibodies (almost 400 specificities are now known) are clinically significant since they do not shorten the survival of red blood cells. Because there is presently no in vitro test that can predict the behavior of antibodies in vivo, a true compatibility test does not exist. A considerable amount of time is spent in countless hospital blood banks on detailed studies of antibodies that are probably benign. It may be possible in the future to determine the clinical importance of antibodies by determining their IgG subclass or by determining

whether they can sensitize red blood cells to the phagocytic activity of macrophages in vitro. As yet, however, no definitive data on these possibilities are available.

Cell Surfaces

The outside of a cell (its membrane) is its "face" to the environment by which it receives and releases information and material. Understanding of cell surface composition and function has been extended remarkably in the past 10 years. Red cells have been important in defining the features of this "face."

Duffy System. It has been demonstrated that entry of one form of the malaria parasite (Plasmodium vivax) into human red cells depends upon the presence of antigens in the Duffy blood group (Fya or Fyb). Individuals whose red cell membranes lack these antigens are protected from infection, presumably because the parasite cannot latch on to a receptor and gain entry to the cell. This finding points the way toward the examination of cell surfaces for receptors for other plasmodia, and affords the opportunity to develop methods for prevention of infection by interfering with parasite-receptor interactions.

Rh System. The complex protein in the red cell surface membrane that confers the Rh blood type is essential to the integrity of the red cell. Absence of the protein in people who have Rh null cells is associated with hemolysis and abnormal cell morphology. Further study of the role of this protein in the integrity of the cell surface should expand the understanding of cell survival and membrane function.

Kell System. This blood group has been shown to be important in red cell membrane integrity and red cell survival and to be related to chronic granulomatous disease (CGD) and certain types of muscular dystrophy. CGD, which is a crippling disease seen in males, is inherited as an X-linked disease. It manifests itself by recurrent bacterial infection that occurs because of an inability of the leukocytes to ingest bacteria. Such ingestion is a necessary first step in killing bacteria. The leukocytes in normal individuals have the kell antigens in their surface membranes; those in victims of CGD have been found to lack them almost entirely. Red cells lacking kell antigens are deformed and do not survive normally. How the absence of kell antigens brings about the structural and functional changes is now under investigation.

Changing Blood Types: Hematological Alchemy. The blood group antigens A and B that determine whether an individual is blood group A, B, O, or AB are characterized by simple sugars attached to membrane proteins. It has been found that a highly specific enzyme can selectively snip off the terminal sugar on B

red cells and thus convert them to O cells. Such transformed B cells (now O cells) have been labeled with radioactive isotopes and injected into other human volunteers where blood is incompatible with type B cells, and instead of being rapidly destroyed, red blood cells survived normally. A comparable specific enzyme is now being sought to convert A cells to O. This transformation of red cells to the "universal donor" type has important implications for practical blood resource management in both military and civil applications.

Program Goals 1982 to 1987

- It is known that various biochemical, physiological, and immunological changes occur in red blood cells during storage, and although significant progress has been made in the field of red blood cell preservation, more basic and applied research is required to characterize and overcome the "storage lesion." It is important to identify the lesion(s) directly responsible for the loss of viability during storage so that additional measures can be taken to further extend shelf-life.

- Efforts should be made to define conditions for maintaining in liquid storage the oxygen-delivery function of red blood cells for the maximum period of time. These studies should evaluate the relative merits of adding anticoagulant preservative solutions directly to whole blood as compared to adding them to the separated red blood cell component.

- Previously frozen, deglycerolized red blood cells closely resemble the ideal red blood cell product. Use of this product is particularly desirable when sensitization to other non-red blood cell components is to be avoided. Simpler freezing and deglycerolization procedures are needed to reduce cost. Although red blood cells can be stored in the frozen state for at least 3 years, the system has been opened and sterility cannot be assured after deglycerolization, and they can be kept at 4°C for only 24 hours. To maintain sterility and prolong post-transfusion shelf-life, the deglycerolization procedure should make use of sterile docking devices. Furthermore, additional research is needed to develop resuspension media that are capable of maintaining function and viability of deglycerolized red blood cells during prolonged liquid storage at 4°C.

- Iron overload syndrome can result from multiple transfusions administered to persons suffering from thalassemia major and other chronic hemolytic anemias. The syndrome

can be prevented by utilizing young red blood cells ("neocytes") for transfusion and removing very old red blood cells ("gerocytes") from the circulation. Efficient methods for separating neocytes and gerocytes from usual red blood cell products, which contain cells of different ages, are needed.

- The question has been raised as to whether current procedures for performing pretransfusion tests for compatibility are cost-efficient and needed. It has been argued by some that less testing would suffice. Blood bank workers have recognized that present techniques fail to detect all antibodies, particularly those having low association constants to their antigens, and that new methods are needed to identify these substances. In addition to improving methods to detect antibody, it is necessary to define the clinical effects, other than overt transfusion reactions, of these molecules. It might be possible to transfuse serologically incompatible, but in vivo compatible, red blood cells to patients without causing ill effects. This practice could lead to more effective use of blood resources. Furthermore, studies in this area would also enable an understanding of the relationship between the structure and function of antibodies.

- Blood group antigens play multiple roles in clinical medicine. Basic studies to define the structure of blood groups and their genetic control should be encouraged. These studies would contribute to a better understanding of membrane function and might help define the physiologic function of blood group antigens.

- Studies to elucidate the mechanism by which blood transfusions enhance survival in kidney grafts should be encouraged. This knowledge may lead to a transfusion therapy that achieves this beneficial effect without eliciting the formation of cytotoxic antibodies.

- The genetic polymorphism of blood groups, red cell enzymes, serum proteins, and leukocyte types provides the basis of testing of paternity. The laboratories that are engaged in these activities, however, do not all perform the range of tests required for a high probability of exclusion. Uniform national standards in paternity testing should be established, and training in accurate procedures should be offered to interested laboratory personnel.

- The use of monoclonal antibodies is revolutionizing laboratory technology. It appears that such antibodies provide stable, reliable, and inexpensive reagents for

cross-matching and typing blood. Monoclonal antibodies might also prove to be invaluable in studying epitopes of blood group antigens and the mechanisms that control immune responses to them.

Research Activities 1982 to 1987

- Expand educational programs for the medical profession to assure proper use of red blood cells in the component form.

- Perfect methods to prepare red blood cells for storage at 4°C for prolonged periods so that when they are transfused, they are viable and functional, and do not elicit, in the recipient, immunization to other blood components.

- Improve methods of freezing red blood cells to reduce cost and to extend the storage period of thawed deglycerolized red blood cells.

- Develop efficient and economical methods to separate neocytes from gerocytes in a mixed population of red blood cells so that they can be used in hypertransfusion programs.

- Improve the cost-efficiency of methods to detect red blood cell antibodies in transfused patients to further increase the safety of red blood cell transfusions.

- Characterize the immunological phenomena responsible for the beneficial effects of blood transfusion on the transplantation of kidneys to improve the planning of transfusion programs for these patients.

- Initiate educational programs for blood banking personnel to improve their skills in HLA typing and serum protein typing. Uniform national standards in paternity testing should be developed and disseminated through workshops.

- Support investigator-initiated research on red blood cell preservation and blood transfusion immunology. To that end, it may be necessary to establish a special study section on blood banking in the Division of Research Grants, NIH.

Platelets

Platelets are the circulating blood cells that are involved in maintaining hemostasis. Platelets accumulate at the site of vascular injury and form a platelet plug. The plug is then stabilized by fibrin, which is formed when the coagulant proteins of blood interact. The major users of platelets are patients with bone marrow depression, usually caused by programs of chemotherapy or radiotherapy given to cancer patients. Alternatively, marrow growth of leukemic cells limits platelet production in some patients with leukemia. In addition, there are some patients with primary bone marrow failure (usually idiopathic) who require long-term support with this product in order to prevent significant hemorrhagic morbidity and mortality. Another large category of patients requiring this product consists of individuals who need massive amounts of transfused blood, such as patients with major traumatic or thermal injuries and those undergoing major surgery. Platelets do not maintain viability in blood stored at 4°C. As a result, patients develop dilutional thrombocytopenia after a significant transfusion requirement, and they need platelet transfusions to maintain vessel wall integrity.

Because of loss of viability and function when stored at 4°C, platelets are separated from blood within 6 hours after phlebotomy. The optimum temperature for storing platelets appears to be room temperature (22°C). At this temperature, however, cell metabolism proceeds, and the process appears to impose limits on the viability of the platelets. In fact, current technology is able to maintain the shelf-life of this cell for only 120 hours. As a consequence, a major problem with this transfusion product is maintaining an adequate shelf inventory.

With the increased availability of this product over the last 10 years as a result of improvements in preparation and storage, there has been a major reduction in the morbidity and mortality of thrombocytopenic patients. A possible adverse result has been the risk of inducing alloimmunization. Since very little is known about platelet antigen systems, alloimmunization to foreign platelet antigens effectively limits the use of long-term platelet therapy. The ability of blood centers to provide compatible platelet transfusions when patients become alloimmunized has been recognized as a major problem.

The National Program in 1972 called for the support of basic and clinical research to improve the collection, preservation, and storage of viable, functional platelets and white blood cells. In addition, clinical research, to be performed in a specialized center of research in blood resources, was called for to improve therapy that uses cellular and plasma components.

Progress in the preservation of liquid and frozen platelets (3 days and up to 6 months respectively) was reported in the Second through the Fifth Reports of the Director, NHLBI. The Fourth and Fifth Reports mentioned new developments concerning HLA matching of platelets.

In addition to these brief comments, the Blood Diseases and Resources Advisory Committee, in its 1976 report, cited the following:

- New information had been obtained on the biochemical, structural, and functional changes occurring during platelet isolation and storage.
- New automated cell-separators had been developed to obtain large-volume, single-donor platelet preparations.

The advisory committee recommended that:

- More research be carried out to characterize the biochemical and physiologic lesions occurring during collection, manipulation, and storage of platelets.
- Clinical studies be undertaken to develop appropriate indications and dosages for platelet transfusions.
- The effects of drugs on platelet survival and function be investigated.
- Improved methods for preparing single-donor platelets be devised.

Progress and State of Knowledge Through 1982

Platelet Availability

Routinely Prepared Platelet Concentrates. During the last decade, platelets became available to an ever larger segment of the patient population. The technology of separating platelets from whole blood was refined, and the method was adopted as a routine procedure. The characteristics of centrifugation were identified that influence the production of an optimal level of in vitro platelet yield and of subsequent in vivo viability.

Single-Donor Platelets. The number of patients who had become alloimmunized to routinely prepared platelet concentrates was increasing, and methods were required that would provide a therapeutic transfusion from a single donor with compatible blood. This problem was solved by a process in which a donor's blood is

collected directly into a spinning centrifuge bowl and the platelets are skimmed off. The remaining plasma and other blood cells are returned to the donor. With this procedure, a therapeutic dose of platelets can be harvested from a single donor in a safe and effective fashion. These platelets maintain normal viability and function when transfused into thrombocytopenic recipients.

Platelet Storage

Liquid Storage. The changes that occur in platelets during storage are now better understood. When platelets are stored at their optimum storage temperature of 22°C, lactic acid is formed as a result of their active metabolic processes, and the pH of the stored plasma drops. A pH below 6 has a deleterious effect on posttransfusion platelet viability and function. Storage of platelets in a relatively large volume of plasma (40 to 60 ml) provides a greater buffering capacity and helps maintain the pH above 6. Gas transport across the wall of the storage bag is also of great importance in maintaining platelet viability, since accelerated entry of oxygen enhances aerobic metabolism. The latter reduces the formation of lactic acid through anaerobic glycolysis. This knowledge has provided an impetus for the manufacture of new types of plastics for platelet storage bags that have extended shelf-life from 3 days to 5 days. When this methodology is adopted nationwide, substantial improvements should occur in platelet inventory management.

Cryopreservation. As with red cells, storage of platelets by cryopreservation is costly. A means therefore needs to be established to identify those patients who are likely to benefit from this product. Currently, cryopreservation of platelets is used to maintain a supply of autologous platelets to manage patients who are expected to become thrombocytopenic and who are refractory to transfusions of platelets of random donors. A few such cryopreservation programs have been established to provide platelets for leukemic patients who are undergoing recurrent inductions for treatment of their underlying malignant disorder. At times of remission, enough autologous platelets are collected to maintain the patient through his next course of chemotherapy and resultant thrombocytopenia.

Current techniques for freezing platelets are much less satisfactory than those for red blood cells, and a number of problems remain to be solved. Various cryopreservatives have been used, such as dimythylsulfoxide (DMSO), glycerol-glucose solution, and hydroxyethyl starch. DMSO has been the most widely used agent during the past 20 years, and it is now licensed for this purpose. Most of the DMSO should be removed prior to transfusion to avoid possible toxic effects (nausea and vomiting, phlebitis, and a

garlic-like odor). In addition, lesions in the eye, particularly in the lens, have been seen in animals given large doses, and DMSO has been shown to potentiate the hepatic toxicity of aromatic hydrocarbons. Healthy volunteers receiving DMSO in the context of previously frozen platelet transfusions, however, have shown no ill effects. Platelets frozen with DMSO have in vitro rates of recovery of about 80 percent after freezing, thawing, and washing, while in vivo recovery rates are between 40 percent and 80 percent of fresh platelets.

A recent improvement in freezing platelets that does not require the concentration of platelets prior to freezing has simplified the procedure. Prepackaged DMSO solution can be added to normal platelet concentrates and the mixture frozen in PVC (polyvinyl-chloride) rather than in polyolefin bags. The thawed platelets can then be washed with buffered 0.9 percent NaCl solution without the addition of plasma.

Platelets with high in vitro and in vivo recoveries can also be frozen with glycerol. Suitable recovery rates, however, have not been confirmed by all investigators.

Platelet Immunology

Platelet Antigens. Although platelet transfusions are effective in nonimmunized recipients, nonresponsiveness develops rapidly as a result of immunization to platelet antigens. By the end of the last decade, it was realized that HLA antigens determine some of the platelet "types" to which patients become immunized. These antigens were of major concern in the development of the nonresponsive state. Anti-HLA antibodies were observed to destroy HLA-mismatched platelets of donors. In contrast, HLA-identical, or even HLA-compatible, platelets were found to be therapeutically effective in some of these refractory patients. Therefore, registries of HLA-typed donors were established in many parts of the country to facilitate the selection of compatible donor platelets for immunized recipients. It has recently been recognized, however, that patients may become alloimmunized even to HLA-matched platelets. Thus, non-HLA platelet antigens exist, and these immunogens may be associated with the development of a refractory state. How to identify these antigens and determine "true" platelet compatibility are major unanswered therapeutic questions.

White Blood Cell Contamination. Studies have indicated that platelet concentrates from which white cells have been removed permit the alloimmunized patient to respond to platelet transfusions. The basis for this observation is thought to be as follows: when given together with white blood cells, platelets are caught as "innocent bystanders" in the reaction between donor

white blood cells and leukoagglutinins in the recipient's serum and are removed from the circulation. It has been shown that the development of nonresponsiveness to platelet transfusions can be delayed if passenger white blood cells in the platelet concentrates are removed prior to transfusion. If these data are confirmed, new methods need to be developed for the preparation of platelet concentrates that contain no, or only a very few, white blood cells.

Platelet Antibody Testing. In recent years, several methods to quantify platelet-bound immunoglobulins have been developed that provide a new tool for studying immunologically mediated destruction of platelets. Such tests may be clinically usable as platelet cross-match procedures.

Program Goals 1982 to 1987

Additional studies are needed in the areas of preparation, preservation, and use of platelet concentrates, particularly the following:

Preparation

- Improved methods should be developed for the collection of platelets of single donors so that larger quantities of platelets can be harvested in shorter periods of time.

- Sterile docking devices should be employed in the procedure of collecting single-donor platelets so that this product can be stored longer than the currently permissible 24-hour period.

- Development of practical methods to remove white blood cells from platelet concentrates without significantly decreasing the numbers of platelets should be encouraged if white blood cells in platelet concentrates are found to contribute to alloimmunization. This effort should result in improved effectiveness of platelet transfusion therapy by delaying the immunization of patients to platelet antigens.

Preservation

- Metabolic and structural changes that occur in platelets during short-term liquid preservation should be more precisely characterized, and changes that are responsible for the loss of platelet function and viability should be

determined. This information should help in the development of improved strategies of platelet preservation and the formulation of better preservative solutions. The result would be a better quality of platelets for patients.

- Cryopreservation of platelets is important in the preparation of HLA-typed platelets for immunized recipients, for stockpiling autologous platelets for leukemic patients, and for inventory control. Currently available methods are reported to produce acceptable in vitro and in vivo recoveries. Upon transfusion, these platelets are hemostatically effective. Such methods should be applied to clinical situations in order to evaluate their usefulness.

Immunology

- Definite characterization of platelet antigens (other than HLA) and antibodies has not been achieved. Furthermore, no acceptable cross-match test for platelets exists. Although individual investigators have used various platelet antibody tests for these purposes, such as radioactive adenine and serotonin release, lymphocytotoxicity, leukoagglutination and platelet aggregation, and radioactive indirect antiglobulin tests, there is no agreement about their value and reliability. Furthermore, clinical evaluation of the patient's responsiveness is hampered by the fact that, in addition to antibodies, other nonimmunological mechanisms can affect the recovery and survival of transfused platelets. A major emphasis should be put on efforts to develop simple, reliable, cost-effective methods to detect and evaluate platelet antibodies.

- In order to facilitate procurement of HLA-matched platelets, the pool of HLA-typed donors should be increased by recruiting persons who have been HLA-typed for other reasons. This purpose could also be achieved by developing a nationwide registry for donors of HLA-typed platelets.

Platelet Use

- Therapeutic strategies most likely to delay or prevent the development of refractoriness to platelet transfusions need to be defined. Planning for patients expected to receive long-term platelet transfusion therapy may, for instance, require red blood cells as well as platelet components from which passenger lymphocytes have been removed as fully as possible. Alternatively, only HLA-matched transfusions can be employed.

- It is necessary to define whether platelet transfusions should be given prophylactically in the absence of bleeding in thrombocytopenic patients. The optimal platelet dosage must also be defined.

Research Activities 1982 to 1987

- Support and encourage investigator-initiated research on the preparation, preservation, and immunology of platelets.
- Encourage research on methods to detect platelet antibodies.
- Initiate multicenter cooperative studies of platelet transfusions to evaluate the best strategies for an optimal transfusion program for platelet recipients.
- Establish a national registry for HLA-typed platelet donors.

White Blood Cells

The 1972 National Program briefly noted that research to improve collection, preservation, and storage of viable and functional white blood cells was needed. According to the program, specialized centers of research in blood resources were a proper setting for undertaking studies related to these subjects. The 1976 advisory committee noted considerable progress in the development of methods for short- and long-term storage of white blood cells and in efforts to understand the significance of leukocyte antigens. The committee recommended the support of research in the following additional areas:

- Leukapheresis procedures for white blood cell donors
- Biochemical and physiologic features of granulocytes
- White cell immunology
- Clinical indications for granulocyte transfusions.

The Fourth Report of the Director, NHLBI, stressed the difficulties encountered with procurement, storage, and viability of granulocytes. The Fifth Report, however, noted that increased yields were achieved using continuous flow fractionation procedures, but that little progress had been made to improve shelf life. The Sixth through Eighth Reports focused on the DBDR studies of the value of prophylactic and therapeutic transfusions

of granulocytes in leukopenic patients. The conclusion reached in the studies was that prophylactic use of white blood cells could not be recommended at that time as standard therapy.

Granulocytes

Since granulocytes are present in the peripheral blood in low concentration, they are more difficult to harvest from whole blood than are other components. Useful quantities can be recovered only by techniques of apheresis, some of which employ premedication of the donor with corticosteroids or add macromolecular agents to the blood during separation. Some collection procedures require heparinization of the donor's blood so that it can be passed through nylon filters, which remove the granulocytes.

Granulocyte transfusions are effective in a sharply defined range of clinical conditions, primarily in patients with gram-negative septicemia complicated by temporary granulocytopenia. Sometimes granulocyte transfusions are complicated by the syndrome of acute pulmonary dysfunction. The cause of this problem has not been fully elucidated, but it appears to be related to complement activation that subsequently leads to granulocyte "activation." The quantity of granulocytes usually transfused is not the same order of magnitude as that provided by the body's own circulating granulocytes under normal physiologic conditions. It appears, therefore, that further progress in this field depends partly on an ability to collect much larger quantities of granulocytes from healthy donors to provide an optimum transfusion dosage.

Granulocyte antigens, which are important in transfusion therapy, have not been characterized. Technological advances in detecting white blood cell antibodies and the process of granulocyte activation are necessary for basic understanding and rational management of granulocyte transfusion therapy.

Lymphocyte Derivatives

Hpman interferons are secretory glycoproteins that are produced by eukaryotic cells in response to viral infection and other stimuli. These proteins are considered a first line of defense against viral infections. They also have other important effects, such as inhibition of cell growth, and antitumor activity. Interferon can also modulate the immune response by enhancing the cytotoxicity of natural killer cells against tumor lines while providing a protective effect on normal cells. The most exciting property of interferons is their possible effectiveness in cancer therapy. Interferons of the alpha, beta, and gamma types are known, the first two being destroyed by low pH. Interferons can be produced by virally induced fibroblasts or

lymphocytes in cell culture. Human lymphocytes, which otherwise might not be used by blood collection agencies, have been a source for large-scale interferon production. Recombinant DNA technology has now succeeded in producing gamma-interferon.

Lymphocytes are also the source for transfer factor, which is one of the more than 130 moieties in the dialyzable leukocyte extract. This product is thought to transmit donor-specific delayed hypersensitivity to a recipient who has not been exposed to an antigen, to stimulate nonspecifically a recipient's capability for delayed hypersensitivity, and to contribute to the stimulation of interferon production in the recipient. For therapeutic purposes, it is necessary to obtain lymphocytes from donors who have proven immunity to the recipient's disease or who have an assumed exposure to the agent responsible for the disease of the patient who is receiving transfer factor.

Stem Cells

Stem cells are the bone marrow progenitors of circulating blood cells. New directions in blood banking involve the collection of stem cells and preservation by freezing. Such a preparation could have great potential in cancer therapy. Allogenic bone marrow stem-cell transplantation is used to treat aplastic anemia and leukemia, whereas autologous bone marrow can be given to rescue the function of bone marrow in patients who have undergone extensive chemotherapy.

Progress and State of Knowledge Through 1982

Granulocytes

During the past decade various methods of apheresis for collecting granulocytes were tested, including nylon filtration and continuous and discontinuous centrifugation procedures. The filtration method was associated with a relatively high frequency of reactions in donors and recipients. Priapism, which occurred in some donors, was severe enough to reduce the popularity of the procedure. Yields obtained by the centrifugation methods could be increased by premedicating the donor with either etiocholanolone or corticosteroids and by using red-blood-cell sedimenting agents such as hydroxyethyl starch, dextran, or gelatin.

Concern has been voiced about the effect on the donor of leukocyte concentrate collection, both in regard to the use of additives to increase the yield of cells, as noted above, and in regard to possible long-term immunologic effects. With respect to the latter, some studies have shown that the proportions of T in

contrast to non-T lymphocyte subpopulations, even in donors who have given as many as 500 times over a 12-year period, remained normal. Likewise, lymphocyte function and surface markers in these donors appear to be unaffected. Others, however, believe that a decrease in long-lived lymphocytes occurs with repeated leukapheresis. No information exists on the possible long-term deleterious effects of depletion of lymphoid cells in relation to the development of malignancy.

The effectiveness of granulocyte transfusions was evaluated in many clinical studies. Granulocytes were shown to be useful as an adjunct to antibiotic therapy in patients with neutropenia and documented gram-negative septicemia. The prophylactic use of granulocyte transfusions does not seem to be beneficial, particularly in view of the number of pulmonary complications experienced by this group of patients. Furthermore, granulocyte therapy is not beneficial for the treatment of febrile illnesses in neutropenic patients when the illness is not associated with positive blood cultures. Septic patients with functionally abnormal granulocytes may benefit from granulocyte therapy. Due to a better understanding of the limitations of therapeutic value of granulocyte transfusions, the use of them is less common now than it was in the beginning of the decade.

Lymphocyte Derivatives

Large-scale production of interferon from human peripheral lymphocytes in Finland made it possible to undertake limited clinical studies to determine the therapeutic effectiveness of interferon in certain malignancies and viral diseases. The use of interferon appears to be beneficial in herpes keratitis, in some respiratory infections, in herpes zoster, in hepatitis, and in osteosarcoma and other malignant diseases. More definitive studies will be possible as more interferon becomes available for clinical use.

Commercial preparation of gamma-interferon by recombinant DNA technology has recently been successful. This methodology should provide sufficient quantities of interferon for large-scale clinical trials.

Transfer factor therapy has been useful in patients having genetically determined broad spectrum T cell defects and in individuals who cannot respond with cell-mediated immunity to certain infections. These infections include those of viral, fungal (chronic mucocutaneous candidiasis), parasitic, and mycobacterial (tuberculosis and its variants) origins. Therapy with transfer factor has been particularly useful when there is no known treatment for the disease, when the patient or his invasive microorganism becomes resistant to standard therapy, or when other

forms of therapy are associated with toxic effects, such as nephrotoxicity. Clinical results have been more consistent when the transfer factor material is tested against the cells of the potential recipient before use and when material of known potency is employed.

The controversy as to whether transfer factor is merely a nonspecific adjuvant or an inducer of cell-mediated immunity de novo has been resolved. Both moieties are present within the dialyzable leukocyte extract. The adjuvant moiety is a peptide with molecular weight of about 12,000, and the antigen-specific moieties are RNA-peptides with molecular weight of about 2,000.

Program Goals 1982 to 1987

Granulocytes

There is a need to encourage scientific investigation in the field of granulocyte collection and transfusion, particularly in the following areas:

- The safety and frequency of administering adrenocorticosteroids and HES to healthy donors before leukapheresis should be studied. Alternative methods for increasing the donor's peripheral granulocyte count should be sought.

- The effects of repeated removal of lymphocytes and stem cells from a donor should be evaluated. On the basis of these studies, it should be possible to develop protocols that ensure safety of donors and to develop criteria for selecting and monitoring donors for leukapheresis.

- No practical guidelines or standards for storing granulocytes exist, although it has been shown that stored granulocytes remain viable and functional after 24 hours in plasma at 4°C. Studies to delineate optimal conditions for storing granulocytes are needed.

- In order to improve the therapeutic effectiveness of granulocyte transfusions, there is a need to develop new white cell collection procedures that provide higher yields of granulocytes than are obtained with present methods.

- Since there is evidence that certain procedures for collecting white blood cells can activate granulocytes and lead to complications for donors and recipients, improved methods of collection are needed. Activation of leukocytes may be initiated by the alternative pathway of

complement activation, but more work is required to elucidate this mechanism. To increase the safety of white blood cell collection and transfusion procedures, it is necessary to develop laboratory tests by which this phenomenon can be demonstrated easily and reliably in blood banks.

- Acceptable methods for testing compatibility of donor's and recipient's granulocytes do not exist. It is not known whether antibodies to HLA antigens or to granulocyte-specific antigens render granulocyte transfusions ineffective, or whether antibodies contribute to the pulmonary complication of granulocyte transfusions. The antibodies that cause chill-fever reactions in recipients of granulocyte transfusions are also not known. A cross-match method is needed that would correlate with the therapeutic effectiveness of granulocyte transfusions.

- Clinical studies that evaluate granulocyte transfusion therapy should be conducted to determine the quantity and frequency of need. The relatively low doses of granulocytes used in a number of previous trials may account for the limited beneficial effects that have been reported. It is also important to explore the effectiveness of granulocyte transfusions in some fungal infections.

Lymphocyte Derivatives

- The recent interest in human leukocyte interferon has created a growing demand for a source for this material. Future recombinant DNA technology may provide large quantities of less expensive interferon for therapeutic use.

- Peripheral blood lymphocytes are the source material for transfer factor. The production of transfer factor requires donors who have proven immunity to the recipient's disease or who have been exposed to agents causally involved in the disease for which the patient is receiving transfer factor.

- Laboratories in blood banks require the capability to measure cell-mediated immunity against various microorganisms in donors. Automated methods to measure production of mediators are needed, as are measurements of active T cells and subsets of T cells. Monoclonal antibodies should prove of great value in developing quantitative methods to determine the amount of transfer factor.

- The mechanism of action of transfer factor remains to be elucidated. To this end, several transfer factors of

human and other mammalian sources should be purified, and their structures defined. On the basis of this information, it may be possible to manufacture transfer factors synthetically in the future. Studies should also be initiated to reveal whether transfer factor is part of the T cell receptor for antigen, as has been suggested. The effectiveness of either immune or nonspecific transfer-factor therapy must be evaluated further on immunosuppressed patients.

Preservation and Collection of Stem Cells

- Infusions of autologous bone marrow have been shown to shorten the duration of myelosuppression after high-dosage chemotherapy in patients with solid tumors. In order to perform multiple procedures of rescue of bone marrow, it is necessary to preserve bone marrow for prolonged periods in the frozen state. In order to obtain higher recoveries of viable hematopoietic stem cells, improved freezing techniques are needed.

- Transplantation of bone marrow has proven to be a useful therapeutic modality in acute leukemia, in aplastic anemia, and in some immune diseases. It is conceivable that in the future this mode of therapy will be practiced more frequently, particularly if it becomes practical to utilize donors outside the family. It is possible that sufficient quantities of stem cells can be recovered from the peripheral blood by techniques of apheresis. This type of collection should be explored because, combined with freezing techniques, it might offer a more acceptable procedure for donors, and particularly for nonfamily donors, than the process of aspirating bone marrow.

Research Activities 1982 to 1987

- Evaluate for donors the safety of procedures for collecting granulocytes, and develop criteria to assess and monitor changes in such donors.

- Improve granulocyte transfusion therapy by exploring new ways to increase the quantity of granulocytes collected from healthy donors and by defining the conditions of storage that lead to the most efficient recovery of viable, functional, nonactivated granulocytes.

- Evaluate the optimum dosage and schedules for granulocyte therapy, and explore the importance of granulocyte transfusions in some fungal infections.

- Develop laboratory tests to enable the physician to assess the compatibility of donor's and recipient's granulocytes.

- Encourage research in collecting stem cells from peripheral blood and preserving them by freezing to support bone marrow transplantation programs.

- Encourage the further processing of lymphocyte-containing buffy coats in order to obtain transfer factor and human leukocyte interferon. Develop capabilities of blood banks for testing specific cell-mediated immunity of lymphocyte donors and for evaluating the potency of lymphokines prepared from these cells.

In order to achieve these objectives, the following general recommendations are made:

- Encourage research in the field of granulocyte transfusion therapy to cover the areas of collection, donor and recipient safety, transfusion immunology, and therapeutic efficacy.

- Encourage cooperation between agencies that collect blood and those that manufacture interferon, to produce large amounts of various types of interferon for clinical trials.

- Support educational workshops for blood bank personnel to enable them to learn methods to measure cell-mediated immunity so that donors can be adequately screened and that those with appropriate characteristics can serve as source material for transfer factor production.

Plasma and Its Derivatives

The 1972 National Program treated plasma fractionation and derivatives somewhat superficially. Its blanket recommendations called for making blood fractionation more efficient and less expensive in order to assure adequate amounts of coagulation factors, and for developing methods to cope with newly emerging demands on the blood resource system. Basic and clinical research, which was most effectively undertaken in the environment of a specialized center of research in blood resources, was to focus on improving component therapy and on developing new methods for protein fractionation.

The First through Fourth Reports of the Director, NHLBI, noted the following progress:

- Improved quality and quantity of factor VIII, eventually leading to the institution of home care regimens for hemophiliacs.
- Improved methods for preparing factor VIII.
- New techniques for plasma fractionation and purification leading to new products and more efficient preparations of known materials.
- The institution of clinical trials of the effectiveness of intravenous gamma globulins.
- The convening of a workshop on the biochemistry, physiology, and clinical uses of albumin.

The 1976 Report of the Blood Diseases and Resources Advisory Committee noted the same developments but pointed out that, in spite of technical advances, cold-ethanol fractionation, in use for over 30 years, was still the most practical method available for plasma fractionation. It recommended support of further research to:

- Develop better separation techniques to improve yield, safety, and cost.
- Learn more about molecular interactions to promote the recovery of minor useful proteins.
- Study genetic polymorphism and immunologic potential of proteins.

The Fifth through Seventh Reports of the Director, NHLBI, documented further progress:

- Purification procedures had been developed to recover most of the factor VIII from cryoprecipitate, leading to the conclusion that demand would be met with little future growth in the supply of source plasma.
- A cooperative study of factor VIII inhibitors included a therapeutic trial that examined the efficacy of prothrombin complex concentrate in treating patients with inhibitors.
- Chromatographic procedures for isolating prothrombin complex, albumin, and gamma globulin were developed.

- An automated plasmapheresis system, using microporous filtration beds, was put into operation.

Plasma

Plasma, the complex aqueous solution in which blood cells and platelets are suspended, may be obtained from whole blood or by plasmapheresis. When derived from whole blood, plasma may be provided in various forms for transfusion or for further fractionation.

Plasma intended for transfusion includes single-donor plasma derived from whole blood and separated from the red blood cells. Single units of plasma may also be used after removal of platelets, cryoprecipitate, or both. Except for certain labile components, plasma is relatively stable during short-term storage in the liquid state. In order to avoid changes associated with liquid-state storage, plasma can be frozen. When frozen solid within 6 hours following blood collection, it is labeled as "Single Donor Plasma, Fresh Frozen."

"Single Donor Plasma, Fresh Frozen," which is a source of coagulation factors, contains almost all of the original factor V and factor VIII activity, although there are more efficient sources of factor VIII. About 80 percent of factor V remains in plasma after removal of cryoprecipitate. The major indication for the use of plasma is to achieve expansion of plasma volume. There are other products, however, that serve this purpose more effectively. Moreover, there is a risk of transmitting hepatitis when plasma is used.

Plasma Derivatives

Plasma derivatives that were available in 1972 and are still in use may be divided into three categories: coagulation products, immune globulins, and plasma volume expanders.

Coagulation Products. Growing knowledge regarding the biological characteristics of some blood clotting factors has greatly contributed to the development of methods for preparing concentrates rich in either factor VIII (antihemophilic factor) or vitamin K-dependent clotting factors. The use of these concentrates is largely responsible for the effectiveness of replacement therapy in deficiencies of clotting factors. Recent progress has been so dramatic that it must now be considered poor practice to use unfractionated materials, except in an emergency, because of the difficulty of achieving adequate and sustained therapeutic levels without inducing circulatory overload. Coagulation products that

were available in 1972 included: cryoprecipitated antihemophilic factor ("cryoprecipitate"), purified antihemophilic factor, and factor IX complex.

Cryoprecipitated antihemophilic factor is a preparation of factor VIII that is obtained from a single unit of plasma collected and processed in a closed system. When plasma is frozen solid (within 6 hours following blood collection) and thawed in the cold, much of the factor VIII coagulant activity (50 to 60 percent) is concentrated in a small amount of poorly soluble material. This cryoprecipitate, which also contains von Willebrand factor, albumin, fibrinogen, fibronectin, factor XIII, and several other proteins, can be separated from the supernatant plasma and used either directly for transfusion or as the starting material for currently licensed AHF concentrates.

Since any blood bank with a freezer can produce a satisfactory factor VIII preparation, the development of cryoprecipitate has revolutionized the management of hemophilia A patients. The main drawback of this material is that it must be stored and shipped in the frozen state. Further, since it is prepared on an individual unit basis, there can be no control of quality of the transfused product. Current FDA regulations require that each bag of cryoprecipitate contain about 80 units of factor VIII coagulant activity. These drawbacks are minimal, and cryoprecipitate has been used successfully in all situations where replacement therapy is needed. One advantage of cryoprecipitate is its ability to provide blood-group-specific replacement and thereby avoid the possibility of hemolytic anemia. Another, and more important, advantage is that it is obtained from a single donor; hence, probability that any one unit is contaminated with infectious agents is low. For the mild hemophiliac who rarely needs replacement therapy, cryoprecipitate is therefore the product of choice. For severe hemophiliacs who are treated frequently, the relative safety of cryoprecipitate (compared with AHF concentrate) represents less of an advantage since such patients are eventually exposed to a very large number of donors (around 500 to 1,000 per year).

Cryoprecipitate contains von Willebrand factor activity, in addition to factor VIII coagulant activity, and this preparation seems adequate to treat both spontaneous and surgically induced hemorrhage in patients with von Willebrand's disease. The product, which contains an average of 250 to 320 mg of fibrinogen per bag, also provides adequate replacement of fibrinogen in those few patients for whom fibrinogen is necessary. Fibrinogen has also been used for replacement therapy in factor XIII deficiency.

In addition to cryoprecipitate, a variety of stable, potent preparations of antihemophilic factor are now available to the clinician. These preparations, which are made from the cryoprecipitate derived from large pools of plasma, vary in degree of purification but not, as far as can be ascertained, in their efficacy for the restoration of normal hemostasis in the hemophilic patient. Available data suggest that the differences in preparation make little difference in the adverse reactions that can occur.

In the manufacture of AHF, cryoprecipitate obtained from pooled plasma is further purified by using several procedures, including an ethanol precipitation step, to yield a lyophilized product. The specific activity of AHF concentrates varies from 0.5 to 3.0 factor VIII units per mg of protein, depending on the procedure of purification. The advantages of AHF concentrates over cryoprecipitate are their lyophilized state, ease of shipment and storage, and a known and labeled factor VIII potency. There are also some disadvantages. Since pooled human plasma representing thousands of donors is the source material for AHF preparations and since the material is not heated to inactivate hepatitis viruses, the risk of hepatitis is present. Anti-A and anti-B blood group antibodies may also be present unless the pooled blood has been specifically tested for them. When large amounts of AHF concentrates are given, these antibodies can cause a mild hemolytic reaction in the recipient whose red blood cells have the corresponding antigen(s).

The advantage of concentrates over plasma is well established for both treatment and prevention of hemorrhage in hemophilia A patients. Raising the level of factor VIII above 25 percent in patients with hemophilia A results in predictably normal hemostasis even during and after major surgery. In surgical patients, factor VIII should be maintained at a minimum level of 25 to 30 percent for 8 to 10 days and at 10 to 15 percent for the next 3 to 7 days. Higher levels (around 50 percent) for a longer period of time should be maintained for patients undergoing orthopedic reconstruction or extensive surgery.

Factor IX complex is a concentrate rich in four vitamin K-dependent clotting factors: prothrombin, factor IX, factor VII, and factor X. Purification methods for the preparation of factor IX complex take advantage of the specific adsorption and elution properties that the vitamin K-dependent clotting factors have in common. The starting material for production is either the supernatant fluid that remains after removal of cryoprecipitate or the by-product (fraction IV-1) that is obtained from the ethanol fractionation of plasma for

the manufacture of albumin and immune globulins. The final product is in a dried state.

Although factor IX complex was initially used for replacement therapy of hemophilia B patients, its availability has been increased and its use has been extended to other clinical conditions. Attention has now been focused on complications associated with its usage, particularly on the transmission of hepatitis viruses. Another reported complication is the occurrence in some patients of thrombotic episodes and disseminated intravascular coagulation. The constituent(s) responsible for these thrombotic complications is not known. Several hypotheses have been put forward: the presence of activated zymogens such as IXa, Xa, and XIa, and the transfusion of considerable amounts of other clotting factors, mainly prothrombin, in addition to factor IX. Compared to hepatitis, the incidence of these complications seems low.

The clinical efficacy of factor IX complex in the management of hemophilia B patients is well recognized, and the availability of such concentrates has brought about a radical change in the therapeutic regimen. Because it is possible to use it in small volumes, it has a distinct advantage over plasma.

Factor IX concentrates have been used prophylactically in hemophilia B patients to reduce the incidence of hemorrhagic episodes and to prevent hemorrhage in the postoperative period. The level of factor IX required in the blood for hemostasis varies with the type of indication and the severity of the lesion. Surgery, for example, requires high doses, and it is generally agreed that factor IX levels should be maintained near 25 to 30 percent. It is of the utmost importance that therapy be continued until healing is complete.

Factor IX complex is also used for replacement therapy of patients with rare congenital deficiencies of other vitamin K-dependent clotting factors. The objective is to obtain in vivo an effective hemostatic level of the missing clotting factor. The clinical efficacy of this concentrate in the management of patients with factor VII and X deficiencies and prothrombin deficiency (or qualitatively abnormal, nonfunctional proteins) is well recognized. In addition, it can be used to treat hemorrhagic episodes and to prevent hemorrhage after surgery. Prophylaxis has also been successful in patients with factor X deficiency.

Factor IX complex has been used in a variety of other clinical conditions including coumarin overdosage, coumarin therapy when immediate reversal of the therapeutic effect of

coumarin is required, and in vitamin K deficiency in adults and in neonates with hemorrhagic disease of the newborn. In addition, it has been used in patients with liver disease, in neonates and premature infants, and in patients undergoing open heart surgery. Except for coumarin overdosage and for reversal during coumarin therapy, there are no clinical data establishing efficacy in these conditions.

No beneficial effects of factor IX complex in open heart surgery have been demonstrated. Because data that are available indicate that the preparation can precipitate disseminated intravascular coagulation in liver disease, many consider the presence of this pathological condition to be a contraindication to its use. Similar complications have been reported in neonates and premature infants.

Although the use of factor IX complex for coumarin overdosage and for reversal of coumarin therapy can certainly benefit the patient, evaluation of the ratio of benefit to risk is of utmost importance since the incidence of transmitted hepatitis is high.

Immune Globulins. Immune globulins, which comprise the second category of plasma derivatives, are isolated from plasma by cold ethanol fractionation. Although immune serum globulin is made from very large pools of plasma and, unlike normal serum albumin and plasma protein fraction, is not heated to inactivate hepatitis viruses, the record of nontransmission of hepatitis by this plasma derivative is remarkable. Specific immune globulins are used to provide passive immunity against bacterial or viral diseases.

Two types of immune globulins for intramuscular administration, available in 1972, are still in use. One of these is immune serum globulin (ISG), which is prepared from plasma pools of at least 1,000 normal donors. Passive immunization with ISG is used for prophylaxis of hepatitis A and for prevention or modification of measles. ISG also provides replacement therapy in persons with hypogammaglobulinemia or agammaglobulinemia. In patients with immunoglobulin deficiencies, ISG may prevent serious infections if circulating IgG levels above 200 mg per 100 ml of plasma are maintained.

The other type comprises the specific immune globulins prepared from plasma containing a high titer of a given antibody or from plasma of hyperimmunized donors. Among the several specific immune globulins used in the past (including measles immune globulin, mumps immune globulin, pertussis immune globulin, vaccinia immune globulin), only Rh_o(D) immune globulin and tetanus immune globulin are currently used. Rh_o(D) immune globulin differs from all other immune globulins with

respect to the purpose for which it is administered. Prophylaxis is not directed toward neutralization of a toxin or an infectious agent, but toward immune suppression. The major indication for Rh_o(D) immune globulin is the prevention of active immunization of Rh-negative mothers who have delivered Rh-positive babies. A second major clinical circumstance occurs when Rh_o(D)-negative patients have accidentally received a transfusion of Rh_o(D)-positive blood.

Tetanus immune globulin is recommended for use after injury if the victim has not been actively immunized (or is incompletely immunized) against tetanus. Tetanus immune globulin has been employed in the treatment of clinical tetanus. Its effectiveness, however, is strongly dependent on the stage of the disease, and the minimum effective dose is difficult, if not impossible, to establish. It is apparently effective in the treatment of tetanus neonatorum, which is a very rare condition in the United States.

Plasma Volume Expanders. Normal serum albumin and plasma protein fraction, which have been available for more than 20 years, are plasma volume expanders derived from plasma. They are prepared by cold-ethanol fractionation and are heated, in the presence of stabilizers, for 10 hours at 60°C to inactivate any hepatitis viruses that may be present.

Normal serum albumin intended for use in the United States is prepared in two concentrations, 5 percent protein and 25 percent protein. The former is used for plasma volume expansion in cases of shock, for postburn therapy, and for correction of protein loss that may follow major surgery. The more concentrated form of the product is used for similar indications and also for correcting osmotic deficits that may accompany gastrointestinal, liver, or kidney disease. Albumin is also used in the treatment of hemolytic disease of the newborn and can be used as the pump-priming fluid for cardiopulmonary bypass.

At least 96 percent of the protein in normal serum albumin must be albumin, as determined by a suitable analytical procedure. Most of this albumin is in the monomeric form, although some oligomers and polymers are formed during heating and subsequent storage. Because its osmotic effectiveness is inversely proportional to molecular weight and because high molecular weight substances can blockade the reticuloendothelial system, it is desirable for the proportion of monomers to be as high as possible to avoid the risk of shock.

Plasma protein fraction, which is always prepared as a 5 percent protein solution, is less purified than normal serum

albumin. The albumin content of plasma protein fraction must be at least 83 percent of the total protein; the remainder consists primarily of alpha- and beta-globulins. Because some of these globulins are less stable than albumin in the presence of heat, the final product contains considerably more polymers than does normal serum albumin.

Plasma protein fraction was originally intended as a plasma volume expander that could be used interchangeably with 5 percent normal serum albumin, but because it was found that plasma protein fraction was much more likely to contain the hypotensive substance prekallikrein activator, as well as other constituents of the kallikrein-kinin system, several restrictions were placed on its use. Plasma protein fraction, for example, is contraindicated for use in cardiopulmonary bypass patients. Furthermore, because of the potential danger of hypotension, plasma protein fraction should not be infused faster than 10 ml per minute.

Progress and State of Knowledge Through 1982

Pathological States Related to Plasma Proteins

In the period from 1972 to 1982, several developments occurred in research on plasma protein deficiency. First, a number of studies focused on the biochemical pathology of congenital diseases associated with known deficiencies of plasma protein, such as emphysema and liver disease associated with alpha-1-proteinase inhibitor deficiency (alpha-1-antitrypsin deficiency), hereditary angioneurotic edema associated with the two forms of C1 inhibitor deficiency (low plasma levels of inhibitor and normal or supranormal levels of nonfunctional inhibitor), and thrombotic states associated with low levels of antithrombin III. These studies resulted in the elucidation of neutrophil proteases in emphysema, complement kinin in hereditary angioneurotic edema, and heparin as an anticoagulant.

Second, some previously known pathological conditions were associated with a deficiency of plasma protein, such as disease states associated with congenital deficiency of individual complement components, delayed clotting associated with deficiency of Fletcher factor (prokallikrein, formerly referred to as prekallikrein), and a combination of factor VIII and factor V deficiency resulting from a congenital lack of protein C inhibitor. Disease states that have been associated with complement deficiencies include syndromes in C1q and C2 deficiencies that resemble lupus erythematosis, syndromes in C3 deficiency that resemble immune

deficiency, enhanced susceptibility to mycotic infections associated with C5 deficiency, and delayed blood clotting associated with C6 deficiency.

Other studies revealed that new disease states were associated with deficiencies of previously unknown proteins such as bleeding due to a congenital deficiency of alpha-2-plasmin inhibitor, impaired generation of kinins associated with a congenital defect in Fitzgerald factor (high molecular weight kininogen), and the syndrome associated with congenital deficiency of C3b inactivator that resembles immune deficiency.

Improvement in Separation of Plasma Proteins

Technology for the production of therapeutically used plasma proteins has been improved since 1972, and a host of new proteins has been isolated. New mild, nondenaturing precipitants have been used for the separation process, including polyethylene glycol for albumin, factor VIII, C1 inhibitor, and antithrombin III; and polyelectrolytes for factor VIII. Mild denaturants such as dithiothreitol have been used for the preparation of intravenous immune globulin and alpha-1-proteinase inhibitor (alpha-1-antitrypsin).

Perhaps the major improvement in the isolation of plasma proteins has been the development of affinity-chromatography techniques. The attachment of nonspecific binding ligands to solid matrices had led to resins with unusual selective properties. The use of zinc chelates, gelatin, mucopolysaccharide lectins, and dyes attached principally to cross-linked agarose gels has yielded new approaches to the separation of plasma proteins such as albumin, the plasma proteinase inhibitors (alpha-1-proteinase inhibitor, antithrombin III, C1 inhibitor, alpha-2-macroglobulin), enzymes (complement proteinases, coagulation proteinases), and binding proteins (fibronectin, C1q complement component, P component of amyloid, C-reactive protein). Similarly, specific adsorption of individual plasma proteins has been obtained with gel-bound plasminogen (alpha-2-plasmin inhibitor), gel-bound retinol (retinol binding protein), and immunoadsorbents (group specific proteins). With the exception of plasminogen and antithrombin III, which are isolated for laboratory use on gel-bound lysine and gel-bound heparin respectively, large-scale schemes for purifying plasma proteins by these methods have not been established. Immunoadsorbents, however, have proved to be useful tools for detecting and measuring plasma proteins and as markers of pathological disease states.

The use of ultrafiltration techniques to concentrate and desalt plasma fractions has also been developed since 1972. This process is now being used routinely in the production of both immune serum globulin and normal serum albumin. Thin film

evaporation, which is a second method for concentrating protein, is also being utilized in the manufacture of albumin.

Improvement of Existing Plasma Derivatives

The introduction of testing for the hepatitis B surface antigen (HBsAg) in 1972 was of major importance for public health. Now performed by methods with considerably higher sensitivity than at the start of the decade, this testing is applied to all blood or plasma, regardless of whether it is intended for transfusion or fractionation. As a result, the major type of posttransfusion hepatitis is no longer hepatitis B, but rather non-A,non-B hepatitis. Furthermore, plasma derivatives are now devoid of detectable HBsAg. Despite this development, derivatives can still be infectious, and additional research is necessary.

A second important advance has been the introduction and increased use of source plasma, which is obtained by plasmapheresis and is used only for the manufacture of plasma derivatives. In most cases, it is frozen immediately after collection and is stored at -20°C or colder. Because more plasma can be collected by plasmapheresis than by single donations of whole blood, a much greater supply has become available, and since freezing preserves factor VIII, all of this plasma can be used for the preparation of antihemophilic factor. As a consequence, U.S. industry has now met--and surpassed--the needs of the U.S. hemophilic population. Moreover, the use of purified antihemophilic factor, which can be stored at room temperature or under ordinary refrigeration, has increased greatly while that of cryoprecipitate, which must be deep frozen, has diminished.

The progressive increase in the availability of AHF has permitted the introduction of home care programs in which the product is self-infused by the patient or administered by someone in the home environment. At the present time, 50 to 80 percent of the hemophiliacs in hemophilia treatment centers are participating in such programs; a small percentage are on prophylactic therapy and the majority are on so-called "early demand" intermittent self-therapy.

Parallel with the increasing use of source plasma came an improvement in the quality, as well as the quantity, of certain plasma derivatives. Immune globulins can sometimes undergo proteolytic degradation during storage as a result of the action of trace amounts of enzymes (primarily plasmin) in the product. One important factor in this degradation is the source of plasma used as starting material. Plasma that is recovered from blood stored past its expiration date (recovered plasma), during which time the zymogens of certain enzymes can be activated, tends to yield less stable immune globulins than does plasma frozen soon

after collection. A second important factor in the degradation is the fractionation procedure itself. The development of useful, though imperfect, tests for predicting the stability of immune globulins has permitted manufacturers to make subtle adjustments in conditions of fractionation that divert proteolytic enzymes and their zymogens away from the final product.

A similar improvement in the quality of plasma volume expanders has come from the recognition of the importance of enzymes of the kallikrein-kinin system in adverse reactions to these products. Since 1977, it has been demonstrated that minute concentrations of prekallikrein activator in the product (10 nanograms per milliliter) can produce hypotensive reactions in recipients. The development of sensitive laboratory tests for prekallikrein activator (a fragment of coagulation factor XII), kallikrein, and bradykinin has permitted not only closer control of these products but also evaluations of processes of fractionation. Analogous to the case of immune globulins, recovered plasma tends to yield plasma volume expanders with higher levels of prekallikrein activator than does plasma frozen soon after collection.

Regardless of the starting material, however, normal serum albumin is much less likely to contain significant amounts of prekallikrein activator than is plasma protein fraction. This finding has led to different instructions for the clinical use of these products.

New Indications for Existing Plasma Derivatives

Cryoprecipitated antihemophilic factor as a replacement for fibrinogen and factor IX complex as a means of bypassing inhibitors (antibodies to factor VIII) are new therapeutic uses of existing coagulation products.

Although fibrinogen was already known to be a major constituent of cryoprecipitate, increased usage of cryoprecipitate as a source of fibrinogen was prompted by the revocation of fibrinogen as a licensed product in December 1977. This revocation was a consequence of the recognition of the minimum need for fibrinogen administration and a significant incidence of hepatitis following its use.

The use of factor IX complex for the treatment of patients with factor VIII inhibitors is more innovative. It is estimated that nearly 10 to 15 percent of patients with classic hemophilia A will develop an antibody to factor VIII. In such patients, therapy for major episodes of bleeding may be effective only if factor VIII is infused in massive amounts or if the level of inhibitor in the plasma is decreased by plasmapheresis or by plasma exchange transfusion before infusion of factor VIII. Despite these

procedures, however, management of the disease constitutes a serious problem when the level of inhibitor is high. The high level of antibody precludes the possibility of successful therapy with AHF concentrates. In these situations, factor IX complex has been used with beneficial effects. The results of a multicenter therapeutic trial (1980) unequivocally established the value of factor IX complex in the treatment of hemarthrosis in hemophilia A patients with inhibitors. The mechanism by which the product "bypasses" the inhibitor is not known. The protein(s) that has this capacity is also not known.

In the area of immune globulins, the use of Rh_o(D) immune globulin, whose primary indication is prevention of postpartum sensitization, has been extended to include administration to Rh-negative women after miscarriage, abortion, and amniocentesis.

New Plasma Derivatives

A new coagulation product introduced in 1981 is the anti-inhibitor coagulant complex (AICC). This product is described as being a "controlled activated prothrombin complex concentrate." Prothrombin complex is a term used by some investigators for factor IX complex. AICC is intended for use only in patients with factor VIII inhibitors who are bleeding or who are to undergo surgery. Although there has been no double-blind controlled clinical trial of the product, its effectiveness in stopping episodes of bleeding is recognized. The mechanism of action by which AICC affects hemostasis has not yet been established, but it is thought to result from activated coagulation factors that bypass the site of action of the factor VIII antibody. This hypothesis has not yet been proven. AICC is prepared from large pools of human plasma and is not subjected to treatment known to diminish the risk of transmission of hepatitis.

New immune globulin products that have become available during the last decade include two types of preparations. One type comprises specific immune globulins for intramuscular use, such as rabies immune globulin (commercialized in 1974), hepatitis B immune globulin (1977), and varicella-zoster immune globulin (1980). These products are analogous to specific immune globulins that were previously available. Their development was not contingent upon major innovations in manufacturing (other than the subtle improvements noted above) but rather upon procedures for obtaining a continuing supply of high titer plasma and upon advances in field, clinical, and laboratory testing.

In contrast, "Immune Globulin, Intravenous," which was introduced in 1981, is the only human immunoglobulin product for intravenous use currently on the U.S. market. Whereas the indication for its use--namely, maintenance of immunodeficient

patients--is the same as that for "Immune Serum Globulin," the process by which it is manufactured is strikingly different. Because of the high incidence of adverse reactions to intravenously administered conventional immune globulins, a wide variety of methods for producing a safe product was explored. The current method of manufacture is limited reduction and alkylation of immunoglobulin G. The advantages of intravenously administrable immune globulin (compared with the conventional product, which is given intramuscularly) are the greater comfort for the patient, the rapid attainment of high levels of antibodies in the circulation, and the option of administering larger doses. The latter advantage is particularly significant when intensive therapy is required or when a patient's musculature is small or damaged.

Clinical Investigation of Plasma Derivatives

Additional proteins of potential therapeutic benefit can be isolated from plasma and be provided to clinicians as concentrates for replacement therapy. Examples of new preparations currently under clinical investigation include the plasma proteinase inhibitors antithrombin III and C1 inactivator, cytomegalovirus immune globulin, and various immune globulins for intravenous use. Genetic defects resulting in diminished synthesis of antithrombin III and C1 inactivator have been clearly associated with thrombotic disorders and angioneurotic edema, respectively. Acquired deficiencies of these inhibitors are also known to occur. Antithrombin III is currently being evaluated in the prevention of thrombotic complications following hip surgery, and a preparation containing large amounts of C1 inactivator is being evaluated for its efficacy in the treatment of acute episodes of angioneurotic edema.

Although cryoprecipitated antihemophilic factor has been available since the 1960's, it is currently being evaluated as a source of fibronectin in patients suffering from conditions such as trauma, sepsis, and burns, in which fibronectin is reportedly decreased.

Program Goals 1982 to 1987

One of the most frequent needs is for reliable statistics on the national production and usage of plasma derivatives. A system that obtains and continuously updates such figures should also monitor the types of usage, such as the clinical conditions for which each derivative is being prescribed.

During the next 5 years, technologies should be developed and improved that assure a continuing supply of needed plasma derivatives, yield derivatives that are free of risk of hepatitis but

retain their structural and functional integrity, provide a supply of useful plasma proteins that are too labile to be isolated by current commercial methods, and lead to safe, effective, and abundant "plasma derivatives" without reliance on plasma as the starting material. At present, some of the most promising technologies are those based on hybridomas. Such technologies and the production of plasma derivatives by recombinant DNA methods should be explored.

An important and critical need is the improvement of communications among the various investigators who are involved in the development of derivatives, and an ongoing mechanism should be devised for identifying derivatives that need to be developed for clinical use. In this respect, the experiences of other countries may be of considerable value, particularly when enthusiasm for new forms of therapy is based on objective clinical evidence of usefulness. To avoid expenditure of time and money for the exploration of unsuitable products, promising new derivatives (and new indications for them) should be brought to controlled clinical trials as early as it is feasible. In pursuit of this goal, however, it must be recognized that a significant change in a manufacturing process may require that a product undergo new clinical investigation. Moreover, a premature or inappropriately conducted clinical trial may prejudice further development of a potentially useful derivative.

Studies of the indications for existing products are needed in controlled settings. Such studies should counter the emergence of "use stagnation," such as situations in which new indications are not investigated or inappropriate ones are continued by habit of established practice. Clinicians should be informed of the outcome of these efforts. An important and continuing need is to apprise the medical community of appropriate uses of plasma derivatives, including information on the characteristics and availability of new derivatives, new indications for existing ones, and indications found to be inappropriate.

Long-term needs include the support of programs that encourage the development of new plasma derivatives suitable for clinical use and that guide research toward a detailed understanding of the functions of plasma proteins.

Coagulation Products

Since pooled human plasma is the source material for AHF, factor IX complex, and antiinhibitor coagulant complex, and since no particular treatment is used to inactivate hepatitis viruses during the manufacture of these products, there is a risk of transmitting hepatitis. Although FDA regulations require that

each donor be tested by FDA-approved methods and be found nonreactive for hepatitis B surface antigen, the effect of this requirement on the incidence of hepatitis B associated with the use of coagulation products is not yet clear. The risk of transmission of non-A,non-B hepatitis, however, is clearly established. There is an immediate need to provide hepatitis-free derivatives for replacement therapy in hemophilia A and B patients. The recent introduction in Europe of an apparently effective and safe, highly purified (equivalent 6 factor VIII units per mg of protein), heat-treated (10 hours at 60°C) AHF concentrate shows that factor VIII can withstand such treatment in the presence of appropriate stabilizers and still retain its biological activity. The main drawback of the method is the substantial decrease in factor VIII recovery in the final product.

Because of the increasing demand for factor VIII worldwide, as well as the increasing cost of this material, it would seem appropriate to support efforts to improve the yields that are obtained in the manufacture of AHF. Other areas of needed improvement include the development of a factor IX complex devoid of "thrombogenic" material(s) and the elucidation of the mechanism by which antiinhibitor coagulant complex bypasses the factor VIII antibody. Identification of the factor(s) responsible for the bypassing activity must be accompanied by the development of laboratory tests for use in monitoring patients treated with this material.

In evaluating the need for the development of new products, consideration should be given to the fact that the only currently available sources of factor V are fresh frozen plasma and fresh frozen plasma from which the cryoprecipitate has been removed (90 percent of the factor V activity is retained in this product). When used appropriately in patients with episodes of bleeding associated with severe factor V deficiency, the benefits of fresh frozen plasma far exceed the risks. Studies to develop a factor V concentrate, however, should be encouraged.

Cryoprecipitate is currently used for replacement therapy in patients with von Willebrand's disease. Since von Willebrand factor can now be successfully separated from factor VIII, it would seem reasonable at this time to assess the need for the development of a plasma derivative that is rich in this protein.

The discovery of the association of a combination of factor VIII and factor V deficiency with a deficiency of protein C inhibitor emphasizes the need for the development of products for replacement therapy in these rare cases. Similarly, the association of thrombotic episodes with protein C deficiency suggests that there is a need to develop a protein C product.

Acute-Phase Proteins and Proteinase Inhibitors

There is still a paucity of information with regard to early acute-phase plasma proteins, such as C-reactive protein and alpha-1-antichymotrypsin. These two proteins increase to very high levels during the first stages of inflammation, but their actual function is still not known. Investigations to elucidate their function should continue, with the long-term goal of producing these proteins as potential therapeutic agents.

The plasma proteinase inhibitors need further investigation, especially since they make up more than 10 percent of the total protein in plasma. There is good evidence that in burn shock and in adult respiratory distress syndrome (shocked lung), the levels of biologically active alpha-2-macroglobulin and alpha-1-proteinase inhibitor decrease in blood and lung fluids. Thus, a strong case can be made for isolating these proteins on a large scale and testing their clinical efficacy.

Immune Globulins

It should be determined whether currently available immune globulin products are being utilized for maximum beneficial effects. Would tetanus immune globulin, for example, be more effective if part of the dose were administered by route(s) other than intramuscular? Does hepatitis B immune globulin have additional applications, perhaps in concert with vaccine? What is the optimal dosage of immune serum globulin for maintaining immunodeficient patients? Should the dosage be the same in primary and acquired immune deficiency and immunosuppression? Some of these questions could be better investigated through the use of "Immune Globulin, Intravenous." In addition, it will be important to learn whether these preparations, which are manufactured by different procedures, are clinically interchangeable.

The role of immune globulins in the treatment, as opposed to prophylaxis, of infectious disease should be determined. This effort will probably require the parallel development of specific intravenous immune globulins such as those directed against particular bacterial or viral antigens. In addition to derivatives currently under evaluation, such products could include intravenous immune globulins with specificity for tetanus, pseudomonas, diphtheria, botulinum, and rubella.

Currently available immune globulins are all of the immunoglobulin G (IgG) type. Recent reports indicate, however, that antibodies which are clinically effective against gram-negative bacteria are of the IgM type. In the development of new derivatives, efforts should therefore be made not only to obtain

products with new specificities but also to determine the feasibility of producing functional IgM on a large scale. Experiences in other countries may again provide useful guidance.

The introduction of "Immune Globulin, Intravenous" in the United States emphasizes the critical need to determine the basis of adverse reactions to intravenously administered immunoglobulin. Failure to understand these reactions has confined the development of these products to early stages of trial and error. Study of the etiology and mechanisms of untoward reactions must be accompanied by the search for laboratory tests that can predict the safety or the hazard of immune globulins for intravenous use.

The use of hybridoma technology to produce therapeutically useful immune globulins should also be encouraged. Particular attention should be given to the development of specific immune globulins that are difficult to obtain by conventional immunization-isolation techniques, and to studies and tests for establishing the safety of monoclonal antibodies.

Plasma Volume Expanders

There is no compelling need to develop additional plasma volume expanders, but in view of the high cost associated with widespread usage of normal serum albumin, there is a strong obligation to establish the positive indications for the product. What, for instance, are the clinical conditions in which albumin has positive beneficial effects that are not provided by electrolyte solutions? Given the availability of 5 percent normal serum albumin and the directives for its appropriate usage, it becomes crucial to determine whether there is any therapeutic importance to "Plasma Protein Fraction."

Other Proteins and Polypeptides

The single most important need is for continued support of basic research on protein chemistry and technologic research on protein separation. Specific areas where initiatives are needed include:

- Isolation and characterization of naturally occurring plasma proteins with specific biological activities, such as histidine-rich alpha-2-glycoprotein, P component of amyloid, fibronectin, and plasminogen activator.

- Identification and isolation of plasma polypeptides with hormonal activity and cell-modulating activity. Platelet-derived growth factor was first identified in serum, even

though it is now recognized as being packaged in the platelet granules.

- Characterization of circulating lipoproteins (apoproteins and nonapoproteins) and their importance to cell membrane functions.

- Determination of whether plasma can be a source for the purification of enzymes for use in replacement therapy in enzyme-deficiency states.

Research Activities 1982 to 1987

- Provide support for monitoring on a national scale the extent and type of clinical uses of plasma and plasma derivatives.

- Develop a program that apprises the medical community of innovations in and appropriate uses of plasma derivatives.

- Create a standing committee to establish research priorities for plasma derivatives.

- Establish an efficient mechanism that permits rapid implementation of clinical trials to determine safety and efficacy of plasma derivatives.

- Initiate clinical studies to establish appropriate indications for the use or exclusion of new and existing plasma derivatives.

- Support fundamental studies of isolation and function of plasma proteins that are, or are likely to become, constituents of plasma derivatives.

- Develop improved procedures for the isolation of existing plasma derivative products to achieve higher yield, better quality, and lower cost.

- Support studies directed to the supply of safe and effective plasma derivatives without reliance on plasma as the source material, such as might be done with hybridoma and recombinant DNA technology.

- Identify the etiology of adverse reactions resulting from the transfusion of plasma derivatives, such as factor IX complex and intravenous immune globulins.

- Support the development of safe and effective products for management of bleeding disorders. Specifically, studies should be encouraged to:

  - Provide sources of factor VIII and factor IX free of hepatitis agents.

  - Provide a source of factor IX that does not induce thrombotic complications and disseminated intravascular coagulation.

  - Identify the factor(s) effective in controlling hemorrhage in patients with antibodies to factor VIII.

  - Support research on von Willebrand factor as a potential plasma derivative.

  - Support research on protein C inhibitor as a potential plasma derivative.

- Support the development of safe and effective products for management of thrombotic disorders. Specifically, studies should be encouraged to:

  - Evaluate the clinical efficacy of antithrombin III.

  - Support research on plasminogen activator and protein C.

- Support investigations of the importance of plasma proteins in inflammatory processes. Specifically, studies should be encouraged to:

  - Determine the function of early acute-phase proteins.

  - Evaluate plasma proteinase inhibitors (alpha-1-proteinase inhibitor, alpha-2-macroglobulin, alpha-1-antichymotrypsin) as potential therapeutic agents in abnormal tissue destruction (emphysema, adult respiratory distress syndrome).

  - Establish the potential of protein replacement therapy in patients who are congenitally deficient in alpha-1-proteinase inhibitor.

- Support investigations of the importance of plasma proteins in therapy of infectious diseases, especially with reference to states of immune deficiency. Specifically, studies should be encouraged to:

  - Establish the optimal dosage of immune globulins, including intravenous preparations.

- Develop and evaluate new specific immune globulins for intravenous use, including those which may consist of IgM.

- Determine the effectiveness of specific immune globulins in the treatment of acute infectious diseases.

- Support investigation of appropriate use of protein plasma volume expanders. Specifically, studies should be encouraged to:

  - Establish the positive clinical indications for normal serum albumin.

  - Strengthen the scientific base that indicates uses of "Plasma Protein Fraction."

Blood Substitutes

Although the 1972 National Program made no mention of blood substitutes, the report of the Blood Diseases and Resources Advisory Committee (1976) noted an increased interest that followed an NHLI-sponsored workshop on the subject in 1974. The committee recommended more basic and applied research on perfluorochemicals, stroma-free hemoglobin, and oxygen-binding chelates.

The Fourth, Fifth, and Sixth Reports of the Director, NHLBI, noted the rapid progress being made in the preparation of new perfluorochemicals and in the development of knowledge concerning the physiologic consequences of partial and total blood replacement. In the Eighth Report, the data being accumulated on the use of stroma-free hemoglobin solutions as blood substitutes were briefly reviewed.

Among the critical requirements that artificial blood substitutes must meet, totally or in part, are transport of oxygen and carbon dioxide, and maintenance of oncotic and osmotic pressure. These requirements have given rise to three major types of artificial preparations. Those of the first type act as plasma volume expanders, such as solutions of dextran, hydroxyethylstarch, and polyoxygelatin. These agents have no special affinity for oxygen and carbon dioxide, and all of them can be produced in a range of molecular weights, which results in different effective periods of retention in the circulation. Originally, high molecular weights (250,000 to 400,000) were favored, but more recently, compounds of less than 100,000 have been preferred. One of the chief uses of these products is short-term restoration and maintenance of circulating volume in emergency situations, where relatively modest total amounts are needed. Larger amounts

interfere with proper hemostasis. Polyoxygelatin has not been used extensively in the United States, and hydroxyethylstarch is apparently employed more often than dextran. There have been reports of low-grade antigenicity with all of these expanders, but the clinical significance of this phenomenon is still not known.

Compounds capable of facilitating transport of oxygen are those that bind oxygen reversibly, particularly chelates and non-polar solvents of oxygen. Such compounds may or may not possess oncotic properties; those that do not can be used in combination with oncotic agents. To date, oxygen-binding chelates of actual or potential biological importance depend upon iron-containing binding sites. Chelates relying on other atoms are often toxic or have inappropriate binding constants. Stroma-free hemoglobin and modifications of it have received the most attention. Outdated human erythrocytes are the source of the hemoglobin, and several procedures are available to prepare the material and simultaneously eliminate the stroma. From the standpoint of purity, it is likely that small amounts of some red cell constituents remain with the hemoglobin even when it is of the "crystalline" variety. Solutions of stroma-free hemoglobin are sterilized by filtration and remain usable for about 6 months when stored at 4°C. There is a longer period of storage if the hemoglobin is freeze-dried and reconstituted just prior to use.

Because hemoglobin is rapidly excreted after intravenous administration, a number of modifications have been made to prolong its period of retention. These modifications involve chemically combining hemoglobin with larger molecules, such as dextran, or cross-linking the hemoglobin molecules to increase the molecular weight. These modifications have the advantage of decreased oncotic pressure combined with retention of the oxygen-binding capacity of the substance. In almost all cases, the modified hemoglobins have tighter binding affinities, which require a lower external P_{O_2} before the oxygen can be released.

Synthetic iron chelates that have reversible oxygen-binding properties are complex molecules. They are generally related to heme, but have various structural features designed to prevent the atom of iron from reacting with more than one atom of oxygen. Because iron chelates are made entirely by synthesis, finished products can therefore be "tailor-made" for the amount of oncotic pressure and other desired properties. To date, several iron chelates have been made that bind oxygen reversibly. They have not yet been tested under physiological conditions, and their immunologic characteristics are unknown.

Perfluorochemicals, which are good solvents for oxygen and carbon dioxide, are being used both clinically and experimentally in preparations for blood replacement. Although these compounds have very low viscosities and surface tensions, they must be

emulsified for intravenous use and for in vitro organ perfusion. They are chemically and biologically inert and therefore form no metabolites. Unlike hemoglobin and other related chelates that actually bind oxygen, perfluorochemicals simply dissolve it. The amount of oxygen that dissolves correlates with the level of P_{O_2}. These preparations are used with an inspired oxygen concentration of 50 percent or more, which is the amount of O_2 normally administered to patients in emergency situations. Perfluorochemical-type blood replacement products provide a high P_{O_2} and release O_2 at any P_{O_2} below that of the preparation, and thereby facilitate the oxygenation of tissues. This class of chemical offers a distinct advantage over chelate-type compounds in which the release of oxygen occurs only when a suitable low P_{O_2} has been reached. Because the emulsions of perfluorochemicals do not possess oncotic properties, hydroxyethylstarch is added along with electrolytes and bicarbonate buffer.

Progress and State of Knowledge Through 1982

Artificial blood substitutes have progressed from what was only an idea a little more than a decade ago to a present-day clinical reality. This advance has broad significance. It shows for the first time that through the use of perfluorochemicals and hydroxyethylstarch, two of the very important functions of blood can be achieved simultaneously without red cells or plasma proteins--namely, transport of oxygen and maintenance of blood volume. In the process, transport of carbon dioxide is also provided for. Thus, new and different ways of approaching many experimental and clinical problems have become available. For patients with special needs, an alternative to blood is now possible. Persons whose religious beliefs forbid administration of blood can take advantage of this development. Patients with requirements for rare blood where no supply is available will be able to rely on an artificial preparation, as will individuals who are refractory to any kind of blood. Major demands can also be met during times of disaster. Preparations that require no typing and are free of infectious agents are ideal for emergency use. For therapeutic procedures using large volumes of blood, an artificial substitute conserves natural blood and its components for special critical needs.

The most significant and rapid progress in this area has been made with preparations that are of the same type as the perfluorochemicals. The perfluorochemicals that are now available leave the body in a reasonably short time and yet make stable emulsions. The compounds are exhaled through the lungs. There is almost no limit to the kinds of preparations that can be made with these compounds. If the concentration of perfluorochemicals in the mixture is increased, the concentration of breathed oxygen can be decreased. No immunological problems have been encountered to

date. More work is needed, however, to develop products that can be stored at room temperatures and that have optimum properties for the particular applications under consideration.

Total replacement of the blood of rats has been accomplished. Such "bloodless" animals not only survived but rapidly regenerated blood cells and proteins. By the time the components of the artificial preparations disappeared, the newly formed blood sustained the animals, which then continued to grow and develop. These results proved beyond doubt that, when properly prepared, this type of artificial substitute can maintain the life of animals in the complete absence of blood and without the subsequent need to administer blood or its components.

Perfluorochemical-type substitutes have been shown to protect animals against high concentrations of carbon monoxide even though this gas is readily soluble in such preparations. Completely exchange-perfused rats behaved normally in 10 percent carbon monoxide for as long as 10 to 20 hours. Even 50 percent carbon monoxide was tolerated for 4 to 5 hours by such bloodless animals. This finding clearly demonstrates that these products can deliver sufficient oxygen to the tissues to prevent interaction of carbon monoxide with cellular constituents such as cytochromes. The results also prove conclusively that the oxygen-carrying function of red cells is not essential when another means of furnishing oxygen is present.

Extracorporeal circulation procedures often require relatively large volumes of blood. Preparations containing perfluorochemicals have been used successfully to offset this need. Animal experiments have shown that high oxygen tensions can be maintained throughout the procedure.

In vitro perfusion studies have shown that mixtures containing perfluorochemicals are effective in maintaining isolated organs such as heart, liver, kidney, brain, and skeletal muscle. Because most of these perfusates contain neither cells nor proteins, debris from such sources has been avoided as the perfusion progresses. Perfusion temperatures have ranged from near 0°C to 38°C.

It was assumed originally that all perfluorochemicals are retained almost indefinitely in the body. Subsequent research has shown otherwise. Perfluorodecalin was found to leave the body at a rapid rate through the lungs. It is now recognized that a number of chemical and physical factors, such as vapor pressure, lipid solubility, and structure, affect the rate at which perfluorochemicals leave the body and also affect the ease of emulsification and the stability of the emulsion. Depending upon the formulation, preparations ranging from cloudy to clear can be produced.

Clinical studies making use of preparations containing perfluorochemicals have been conducted in Japan, Europe, and the United States. The preparations that were used contained an emulsion of perfluorodecalin, which is a stabilizing agent, and perfluorotripropylamine. Nevertheless, it was still necessary to keep the emulsion frozen. Hydroxyethylstarch was used as the oncotic agent. More than 300 patients have received the material for a variety of hemorrhagic and nonhemorrhagic problems. The quantities that were administered ranged from a few hundred milliliters to as much as four liters.

Reactions, such as an increase in serum GOT levels, were observed in a limited number of patients, but none were considered serious. Thrombocytopenia was reported in a few cases. Increased venous P_{O_2} and O_2 consumption values indicated that oxygenation was effective. Most patients breathed 50 percent O_2 or higher, and in some instances 100 percent O_2 was used, especially during operations. Many of these patients would have received O_2 anyway. In some instances, use of the artificial product was obviously lifesaving.

Because of the increased P_{O_2} values obtainable with the perfluorochemical preparations, their use in treating tissue anoxia has been under investigation. In heart anoxia experimentally induced in dogs, the areas of postanoxic damage were significantly reduced when a perfluorochemical emulsion, rather than blood, was administered. In other studies perfused hearts subjected to anoxia responded better to cardioplegic solutions containing emulsified perfluorochemicals. In experiments on brain anoxia, progress was more satisfactory when emulsified perfluorochemicals were used during recovery.

Research with stroma-free human hemoglobin has been conducted by many scientists during the past 10 years. Whereas in earlier studies the end product was always solutions of hemoglobin, the newer materials, such as "crystalline hemoglobin," are obtained as freeze-dried products. These are reconstituted into solution prior to use. The dried material can be stored for long periods with relatively little deterioration. The absence of stroma prevents the kidney damage that can occur when hemoglobin is given intravenously. It may be possible now to learn definitively what effect, if any, free hemoglobin itself has on tissues in vivo.

Stroma-free hemoglobin itself has a period of circulatory retention of about 3 hours. As a consequence, considerable research has been addressed to producing modified hemoglobins that will stay in the circulation longer and still be able to release oxygen. Dextran-hemoglobin complexes have been produced that have very good periods of circulatory retention, but the P_{50} values are less satisfactory. Nevertheless, dogs breathing normal air survived when exchange-perfused with these materials until their

hematocrits measured about 1 volume percent. Their venous P_{O_2} values were very low. These findings indicate that survival does not depend upon maintaining an oxygen tension that is ordinarily considered satisfactory.

Another strategy for keeping hemoglobin in circulation is to increase molecular weight and size by linking the hemoglobin molecules together. In this way, the O_2 binding sites are not diluted by the introduction of large molecules such as dextran. Furthermore, these polyhemoglobins possess less oncotic pressure to the extent that concentrated solutions can be used without risk of increasing the blood volume. Baboons exchange-perfused with such preparations survived for the duration of the experiment, usually several hours. Release of oxygen was essentially the same as for hemoglobin. The metabolic consequence and the antigenicity of these compounds are not known.

Synthetic iron chelates that bind oxygen reversibly have structures related to heme. The three means of protecting iron from irreversible oxidation are generally classified as "capped," "strapped," and "picket fence" structures. A large amount of experience has been gained in the complex synthetic chemistry involved, and several compounds have been made that reversibly bind oxygen. Although they are not yet suitable for use in blood substitute preparations, it is likely that success will soon be forthcoming in this area.

Program Goals 1982 to 1987

Since many of the needs for blood are associated with its ability to transport oxygen and to maintain osmotic pressure, artificial substitutes that can furnish these functions are attractive alternatives. That such substitutes are effective has now been demonstrated experimentally and clinically. Continued basic and applied research on the preparations themselves and on their experimental and clinical use is essential.

The perfluorochemicals, emulsifiers, and plasma volume expanders that are currently available must be studied with respect to the best possible combinations to achieve preparations that are stable and that have high concentrations of the fluorocompounds. The higher this concentration is, the lower the level of oxygen is required. At the same time, a low viscosity must be maintained. Such studies will require basic and applied scientists with experience in the formulation, preparation, and testing of dispersed systems. It would also be desirable to synthesize a limited number of perfluorochemicals related to the most promising ones now on hand. Relatively small modifications in structure can significantly alter the stability of an emulsion and its period of retention. This activity is quite different

from that of earlier programs, which concerned the synthesis of perfluorochemicals to make many different compounds, in hope of finding a few with great promise.

Of considerable importance is the development of emulsifiers that are effective and are safe for intravenous use. Pluronic F-68 and phosphatides, which are presently available, have allowed the production of a number of finely dispersed perfluorochemical emulsions, several of which are produced commercially. Some promising fluorocompounds, however, cannot now be suitably emulsified. New emulsifiers would help the development of preparations using these agents and might also allow the use of higher concentrations of the more common perfluorochemicals. Different emulsifiers could also influence the viscosity, flow, and period of circulatory retention of the emulsions.

Where possible, basic research on the biological effects of the perfluorochemical preparations should be conducted on a number of species, including humans. Studies should be undertaken to assure the complete safety of the products. It would be of considerable interest and importance to ascertain the reasons for the lack of bleeding of totally exchange-perfused animals and to learn the basis of the rapid hematopoiesis that occurs in the absence of elevated erythropoietin levels and in the presence of adequate circulating oxygen. A number of other intriguing questions remain to be answered.

With the availability of both solvent and chelate types of carriers of oxygen, the biological study of this essential gas will be accelerated. Where oxygen is now used for therapeutic purposes, the proper choice of carrier will improve its effectiveness. Perfluorochemical preparations, for example, should be helpful in treating carbon monoxide poisoning because the preparations can transport oxygen to the tissues and transport carbon monoxide from the tissues to the lungs. If hyperbaric oxygen treatment is required, the presence of emulsified perfluorochemicals in the circulation should greatly increase the effectiveness of the oxygen and allow for use of lower chamber pressures.

Organ perfusion has become increasingly important, and research concerning long-term perfusion should be supported. The substitutes that are free of proteins and cells will cause less debris to form during perfusion, and the preparations that depend on solubility of O_2 ratner than on chelation will have advantages when low perfusion temperatures are required. Perfluorochemical preparations for kidney preservation have been used clinically with subsequent successful transplantation into a human recipient. It would be extremely useful to develop perfusion media that would sustain organs for many days.

Since stroma-free hemoglobin has not yet reached a stage for clinical trials, emphasis in this research area must be placed on nonhuman subjects. Detailed animal tests designed to evaluate solutions of this material in partial and total blood replacement are needed. If the animal tests yield encouraging results, human trials should be undertaken. If the animal tests are not encouraging, the relative importance of further studies with stroma-free hemoglobin should be evaluated. Regardless of the outcome of the research on hemoglobin itself, basic studies to produce modified hemoglobins should continue.

Synthetic oxygen-binding chelate research should be pursued at a basic level to furnish materials for both chemical and biological study. The products developed under such a program will need to be evaluated for safety and efficacy under normal physiological conditions. Work in this area will furnish fundamental information concerning oxygen binding as well as compounds for ultimate use in blood substitutes.

Improved plasma volume expanders are needed, especially ones that have low viscosities and that can be administered in large amounts. This effort will require concepts and compounds that do not now exist.

With increases in clinical applications of blood substitutes, it has become important to train personnel in the uses of these materials. Training additional basic researchers in the field of blood substitutes should also be pursued. PhD's as well as MD's should be encouraged to enter this area of research. Consideration should also be given to training predoctoral students. Similarly, the medical profession should be educated as to the availability, use, and relative advantages and disadvantages of these products. Since the public is deeply involved in blood programs, factual information concerning blood substitutes should be made available.

Research Activities 1982 to 1987

- The overall goal of in vitro and in vivo research on artificial blood substitutes is to obtain safe preparations that effectively transport oxygen and carbon dioxide and maintain blood volume. They should not interfere with normal biochemical or physiological functions such as clotting, immunocompetence, and wound repair.

- Much progress has been made in the development of perfluorochemical-type blood substitutes for both experimental and clinical use. A goal of future work must be to have a larger pool of compounds on which to draw in order

to make it possible to insure preparations that are suitable for specific applications.

- Stroma-free human hemoglobin and modifications of it provide both oxygen transport and colloid osmotic pressure in the same molecules. It is essential to demonstrate conclusively that these products are safe and effective.

- The search for synthetic oxygen-binding chelates that react reversibly with oxygen should continue. In addition to having great potential for use in substitutes for blood, such compounds can be used to expedite the understanding of oxygen chelation itself.

- No ideal artificial plasma expander has been developed to date. Plasma expanders that have low viscosities, do not affect blood clotting and hemostasis, are immunologically unreactive, and have moderate periods of circulatory retention should be pursued.

Specific efforts in the blood substitute area are:

- Wherever possible, training of both predoctoral and postdoctoral personnel in basic and applied research in the field of artificial blood substitutes should be encouraged. This training should include developmental, metabolic, and clinical aspects of blood substitutes studied in university, blood bank, and hospital environments.

- Research concerning the perfluorochemical-type blood substitutes should be encouraged at both the basic and applied levels. Comprehensive animal studies dealing with safety and effectiveness should be undertaken. When feasible, clinical investigations should be undertaken to evaluate preparations in a variety of patients. Since the number of emulsifying agents suitable for intravenous use is small (only two presently), research directed toward the synthesis of new emulsifiers should be supported.

- Comprehensive animal studies with stroma-free hemoglobin should be encouraged. If the results warrant it, clinical protocols should be developed for testing the material in patients, and clinical studies should be undertaken to evaluate the hemoglobin.

- The development of modified hemoglobins, such as polyhemoglobin, should be encouraged. Any of these compounds that withstand rigorous testing should be considered for clinical trial at the appropriate time.

- Research should be encouraged on synthetic chelates that bind oxygen reversibly.

Safety of Blood Donors and Recipients

Safety issues were an important concern of the National Program of 1972. Transfusion-transmitted hepatitis, sensitization, and human error were discussed. The use of autotransfusion to increase safety and, at the same time, reduce demand on the nation's blood resources was considered. The 1972 recommendations included:

- More sensitive tests for hepatitis carriers be perfected.
- Methods of passive and active immunization against hepatitis be developed.
- Studies to eliminate cytomegalovirus transmission be supported.
- The use of autotransfusion be explored.
- A fail-safe system for donor-recipient identification be worked out.

Advances in the field of posttransfusion hepatitis related to hepatitis B virus transmission dominated the first four reports of the Director, NHLBI. The following developments were noted:

- A liaison committee with representation from all relevant government organizations was established to exchange information and advise on problems related to hepatitis. This committee was later replaced by the NIH Viral Hepatitis Coordinating Committee.
- Epidemiologic studies were undertaken relating to hepatitis B infection and to cytomegalovirus infection in the posttransfusion state. Alternate methods of transmission of HBV were discovered.
- The efficacy of HBV immunoglobulin was evaluated.
- The chimpanzee was shown to be useful in hepatitis research.
- Techniques to inactivate HBV were developed.
- By the latter part of the decade, most transfusion-transmitted hepatitis was found not to be related to

either hepatitis A or B viruses, but to new agents called non-A,non-B.

- The serologic changes associated with hepatitis were chronicled.

In addition, the following problems of safety were reported:

- Methods for fail-safe identification of donors and recipients were actively pursued inasmuch as clerical errors remained a major source of difficulty in blood transfusion therapy.

- Sterile docking devices, particularly for use in relationship to thawing of frozen red cells, were further developed.

- The leaching of plastics from blood bags into blood was determined to be a significant problem.

The 1976 advisory committee report reiterated the advances noted above and added new developments:

- Progress has been made toward development of a HBV vaccine suitable for active immunization.

- A chimpanzee colony had been established for hepatitis research.

- A systematic collection of sera from non-A,non-B hepatitis cases was initiated.

- Maternal-fetal transmission of HBV was proven.

- Observations were made that CMV and toxoplasmosis, while transfused quite often, produce diseases only in special groups of recipients.

- A uniform, machine-reading, labeling system was considered to be the only practical device for reducing human and clerical errors in transfusion therapy.

The advisory committee suggested that a new emphasis be placed on understanding the epidemiology and clinical course of non-A,non-B hepatitis, that serologic tests for the virus(es) be developed, and that methods for removing virus from the blood are needed. In addition, the committee recommended further work on inert, nontoxic surfaces for blood bags, automated typing and cross-matching techniques, and methods for fail-safe identification of patients.

The Fifth through Eighth Reports of the Director, NHLBI, continued to document progress in these areas and to emphasize hepatitis research. In particular:

- Purification of HBV led to a practical vaccine that was subsequently demonstrated to be highly effective.
- A detailed examination of the serology of HBV infection led to a new radioimmunoassay for the antigen.
- HBV was found to be transmitted in frozen red blood cells.
- Attempts were made to interfere with the maternal-fetal transmission of HBV by using hepatitis B immune globulin.
- The incidence of non-A,non-B hepatitis was shown to be high--about 90 percent of all posttransfusion hepatitis being due to this cause. In the absence of serologic tests for the agents involved, elevated alanine aminotransferase levels were considered to be helpful indicators of the presence of the virus.
- Studies of the epidemiology and serology of non-A,non-B hepatitis were undertaken.

TRANSFUSION-TRANSMITTED HEPATITIS

One of the major complications of blood transfusion is the transmission of viral hepatitis from donor to recipient. While differences between the clinical syndromes of types A, B, and non-A,non-B viral hepatitis become apparent on analysis of a large number of cases, variations in patterns of onset and course are not reliable for the diagnosis of individual patients with icteric viral hepatitis.

Hepatitis A is frequently heralded by nonspecific symptoms such as fever, chills, headache, fatigue, generalized weakness, and aches and pains. A few days later, anorexia, nausea, vomiting, and right upper abdominal pain appear, followed closely by dark urine and light stools, and jaundice of the sclera and skin. Because of the transient viremia that is most often associated with clinical illness in the donor, this form of hepatitis is rarely transmitted by blood.

The prodrome of hepatitis B and non-A,non-B hepatitis is often prolonged and more insidious. The well-known clinical features of the icteric phase are similar for types A, B, and non-A,non-B viral hepatitis. Biochemical test values indicating liver disease are also similar for all three types, although the serum enzyme and bilirubin elevations tend to be more prolonged in

hepatitis B and non-A,non-B hepatitis. With the exception of hepatitis B after blood transfusion, the mortality is low (about 1 per 500 to 1,000). Higher mortality rates have been reported in some countries for hepatitis occurring during pregnancy. The latter cases have often been considered to be hepatitis A, but the causative agents need to be further investigated. Hepatitis A does not appear to cause the persistent or chronically active hepatitis usually associated with hepatitis B or non-A,non-B.

In view of the difficulty of differentiating these diseases on clinical and biochemical grounds, the advent of specific serological diagnostic tests for infection by both hepatitis A and hepatitis B viruses has assumed great importance. These procedures in combination with specific serological tests for the detection of infection by Epstein-Barr virus and cytomegalovirus have revealed the existence of a category of disease that results from none of these agents and is referred to as non-A,non-B hepatitis. Although a diagnosis can be made only by exclusion, a considerable amount of information is available about the epidemiology of the disease. Non-A,non-B hepatitis, which has a number of features in common with hepatitis B, has been detected in every country in which it has been sought. Non-A,non-B hepatitis is commonly recognized as a complication of blood transfusion, and in countries in which HBsAg-positive blood donors are excluded, it may account for up to 90 percent of all cases of posttransfusion hepatitis. In the United States, the incidence of posttransfusion non-A,non-B hepatitis among patients receiving 5 or more units of blood varies from 11 to 18 percent depending on the source of the blood and the number of units administered. The disease has also been associated with the administration of clotting factor concentrates or washed erythrocytes.

In most communities, a significant number of cases do not appear to be associated with transfusions. Such "sporadic" cases have been found to account for as much as 25 percent of all adult viral hepatitis in some areas. Non-A,non-B hepatitis has also been reported in settings in which the percutaneous transmission of etiologic agents of viral hepatitis is likely, such as hemodialysis, units of heart or renal transplantation, and illicit use of drugs. In general, the disease is mild and often subclinical. There is evidence, however, that infection may be followed by prolonged viremia and the development of a carrier state. Several recent studies of the histopathological sequelae of acute non-A,non-B infection suggest that chronic hepatitis may occur in as many as 50 percent of patients. Persons with hemophilia are particularly at risk of contracting non-A,non-B hepatitis since they receive repeated infusions of clotting factor concentrates, primarily antihemophilic factor and factor IX complex.

Progress and State of Knowledge Through 1982

Pathophysiology of Hepatitis B Virus. Hepatitis B infection leads to the appearance in the serum of hepatitis B surface antigen (originally referred to as Australian antigen) during the incubation period. The appearance occurs 2 to 8 weeks before there is biochemical evidence of liver dysfunction or the onset of jaundice. This antigen persists during the acute illness. In uncomplicated acute cases of hepatitis B with recovery, the antigen is usually cleared from the circulation during convalesence. About 5 to 10 percent of acute cases progress to chronicity, in which both HBsAg and hepatitis B virus continue to circulate in the blood (chronic viremia). Circulation of hepatitis Be antigen (HBeAg) in association with the core or nucleocapsid of the hepatitis B virus precedes serum aminotransferase elevations. Antibody to the hepatitis B core antigen (anti-HBc) is found in the serum 2 to 4 weeks after the appearance of the HBsAg, and it is always detectable during the early acute phase of the illness. Anti-HBc IgM becomes undetectable within 6 months to 2 years, depending on the sensitivity of the test used for its detection. Anti-HBc IgG may remain detectable for many years and possibly for life.

Antibody to HBeAg (anti-HBe) usually appears next. Antibody to HBsAg (anti-HBs) appears last, during late convalescence. It is the antibody usually associated with immunity to reinfection. Precipitating antibodies that react with complete hepatitis B virus particles (anti-Dane antibodies) have been described. They may have an importance in the clearance of circulating HBV and the termination of an acute infection, and their absence in patients with chronic active hepatitis might explain why the infection persists in such patients. In addition to the various serological markers that appear during the course of uncomplicated infection, cell-mediated immune responses to hepatitis B antigens have been described.

The hepatitis B virus carrier state is serologically characterized by persistence of HBsAg and, in most cases, the absence of detectable anti-HBs. Such carrier states are associated with liver damage that can range from minor changes in the nuclei of the hepatocytes to chronic active hepatitis and cirrhosis. Factors have been identified that increase the risk of developing the carrier state. It is more common in males, more likely to follow infections acquired in childhood, and more likely to occur in patients with natural or acquired immune deficiencies. Seroepidemiological surveys reveal that there is a large, worldwide reservoir of persistent carriers of hepatitis B virus. The estimate ranges from 150 to 200 million. Anti-HBc is present in chronic carriers, often in high titer, and there are

reports that anti-HBc IgM remains detectable in some carriers. In some hepatitis B carriers, specific, usually fluctuating DNA polymerase activity remains, and HBeAg persists; in others, anti-HBe is present. HBeAg has been reported to be commoner in younger than in older carriers, while the number of people in whom anti-HBe is detectable appears to increase with age.

A close correlation has been demonstrated between the presence of specific DNA polymerase and infectious hepatitis B virus. The detection of specific DNA polymerase is therefore a useful marker of HBV replication. This enzyme activity is often found in the course of infection when large numbers of virus particles are present. It generally correlates with the presence of HBeAg, and it persists in some chronically infected individuals with continuing viral replication.

HBsAg Screening. The development of serological tests for hepatitis B has permitted the identification of almost all donors whose blood is capable of transmitting hepatitis B to recipients who are susceptible (seronegative) by transfusion. HBV coexists with HBsAg in varying ratios in different individuals. Strongly HBsAg positive serum can contain as many as 10^{12} to 10^{14} particles per ml. The HBV:HBsAg ratio may be as high as 1 to 200. Current sensitive methods to detect HBsAg can detect concentrations as low as 10^{8} to 10^{9} particles per ml. Other serological markers have been associated with high or low HBV:HBsAg ratios, but regardless of the acute or chronic nature of the infection, the detection of HBsAg is strong evidence that viable HBV is present unless inactivating procedures or materials have been applied.

A number of prospective studies of transfused patients have documented a significant decrease, up to between 85 and 90 percent, in the incidence of hepatitis B after the transfusion of blood that was negative for HBsAg by sensitive serologic tests such as radioimmunoassay. Studies have shown that blood that is negative for HBsAg by less sensitive tests (counterimmunoelectrophoresis) and that is positive by more sensitive tests (radioimmunoassay) can transmit hepatitis B to susceptible recipients. Similarly, HBsAg positive sera diluted beyond detection by even the most sensitive test for HBsAg can still transmit hepatitis B. Despite the use of the most sensitive tests to detect HBsAg positive blood, some cases of hepatitis B continue to occur. Approximately 10 percent of the posttransfusion hepatitis in the United States remains hepatitis B despite the elimination of all blood positive for HBsAg by radioimmunoassay.

HBsAg testing has resulted in the identification of a large group of prospective blood donors in whom HBsAg can be detected ("high-risk donors"). Hepatitis B, as well as non-A,non-B hepatitis, transmitted by high-risk donors remain significant problems. Steps to identify such donors and to prevent the transfusion of their blood may provide the most effective means, other than specific serological testing, to reduce further the risk of posttransfusion hepatitis due to HBV. The risk of hepatitis B posttransfusion hepatitis increases when there is:

- Inadequate testing for HBsAg
- Failure to identify and exclude blood from high-risk donors
- Large numbers of units of blood transfused into a single patient
- Transfusion of blood products or plasma derivatives produced from large pools of plasma.

Other Screening for Hepatitis B Virus. It has been suggested that the detection of anti-HBc in high titer or anti-HBc IgM in the absence of both HBsAg and anti-HBs may identify individuals who circulate HBV. This observation needs scientific confirmation. Poorly controlled, retrospective studies have implicated units of such blood (positive for anti-HBc, negative for HBsAg and anti-HBs) in the transmission of hepatitis B to recipients. In other studies, as many as 3 percent of all blood donors and 6 percent of those over 40 years of age have anti-HBc in the absence of either HBsAg or anti-HBs. To date, attempts to transmit hepatitis B to susceptible nonhuman primates with the use of blood positive only for anti-HBc in high titer (including two implicated samples identified in one of the retrospective studies cited above) have not succeeded. This failure could be due to the relative paucity of HBV (dose related), the noninfectivity of such blood, or the absence of infectious HBV from some sera.

Passive Immunization by Hyperimmune Globulin. The major indication for the administration of hepatitis B immune globulin is a single acute exposure, such as occurs with accidental inoculation, contamination of the conjunctiva, or ingestion of HBsAg positive material. Doses in the range of 0.04 to 0.07 ml per kg have been used with success. On the basis of available data, two doses of hepatitis B immunoglobulin administered 30 days apart are required for efficacy. If hepatitis B immunoglobulin is not available, the administration of immune globulin should be considered.

Active Immunization by Vaccine. While most viral vaccines are derived from viruses grown in tissue culture, hepatitis B virus has not been successfully isolated and cultivated in vitro. Therefore in order to prepare a hepatitis B vaccine, it has been necessary to rely on other sources of viral antigen. Plasma of asymptomatic individuals who are chronically infected with hepatitis B virus provide a readily available source of noninfectious hepatitis B surface antigen, which is specifically the 22 nm spherical and filamentous forms of HBsAg that represent excess virus surface material synthesized by infected liver cells but never assembled into complete viruses. This viral antigen, which lacks the hepatitis B core antigen, the DNA-dependent DNA-polymerase activity, and the double-stranded circular DNA that characterize mature hepatitis B virions, is noninfectious. The noninfectious material can be separated from the infectious particles by biophysical means. The spherical forms in particular can be highly purified in large quantities from the plasma of asymptomatic chronic carriers of HBsAg. Free of detectable liver cell components or host proteins, appropriately inactivated spherical forms serve as "subunit" vaccine. Such a subunit vaccine, shown in clinical trials to be 96 percent effective, was licensed in the United States on November 16, 1981.

Acute infection with HBV is a major public health problem, as are chronically infected individuals. The high rate of infection with HBV among certain groups in developed countries and its endemicity in many developing countries indicate a major need for an effective hepatitis B vaccine.

Non-A,Non-B Hepatitis. Animal studies conducted in 1978 demonstrated that chimpanzees are susceptible to infection with several strains of non-A,non-B hepatitis. The disease has been transmitted with a variety of inocula, including serum or plasma from patients with acute or chronic infections and certain lots of clotting factor concentrates. Incubation periods (measured to the first significant elevation in alanine aminotransferase) range from approximately 2 to 14 weeks. Like humans, some chimpanzees also appear to become chronic carriers. Plasma obtained from one chimpanzee 16 months after inoculation with antihemophilic factor was still infectious. Many of the infected animals continue to have elevated alanine aminotransferase activity for months or years.

Clinical, epidemiological, and experimental studies suggest that non-A,non-B hepatitis may be caused by two or more infectious agents. Clinical evidence is based on the observation of multiple attacks of hepatitis in individual

patients. Epidemiologically, short-incubation and long-incubation forms of non-A,non-B hepatitis have been described, although it is possible that this distinction reflects differences in the infective doses. Experimental evidence for the existence of two distinct non-A,non-B hepatitis agents has come from recent cross-challenge studies in chimpanzees.

Non-A,non-B hepatitis in chimpanzees is characterized by the formation of unique cytoplasmic structures, and these changes appear to "breed true" when the infectious agent is cultivated in vitro. Changes in the morphology of hepatocyte nuclei also occur, including the formation of irregular nuclear membranes and the appearance of clusters of 20 to 30 nm particulate intranuclear structures.

Viral-like particles with diameters of approximately 27 nm have been found in antihemophilic factor preparations shown to induce non-A,non-B hepatitis in intravenously inoculated chimpanzees. Identical particles have been recovered from the acute-phase liver obtained from these chimpanzees. Other investigators have identified particles of varying sizes and shapes in specimens obtained from patients and chimpanzees with non-A,non-B hepatitis. None of these findings, however, have been confirmed.

Several investigators have reported the development of serological procedures for an antigen and antibody specifically associated with non-A,non-B hepatitis. These include double immunodiffusion (agar gel diffusion), counter-immunoelectrophoresis, radioimmunoassay, and immunofluorescence. None of these procedures have, as yet, proved reproducible.

Several studies of the efficacy of immune globulin for the prevention of posttransfusion hepatitis have been conducted. There is currently no clear evidence of the efficacy of these preparations in preventing non-A,non-B hepatitis. Until it becomes possible to assay the level of specific antibody in individual globulin preparations, a need for further studies is not indicated.

Program Goals 1982 to 1987

Particles and In Vitro Tests. A variety of virus-like particles have been described in chimpanzee and human liver or sera in non-A,non-B hepatitis as well as in clotting factor concentrates. These particles can be explained in several ways. There may be a variety of particles associated with hepatitis B virus. Non-A,non-B hepatitis may be caused by a number of different agents with different morphological

appearances. Some or all of the described particles may be unrelated to the agent or agents themselves but be related to the disease. Until a single particle can be described in all, or at least in a defined group of cases of non-A,non-B hepatitis, no conclusions can be made regarding the specificity of particles so far described.

Although relatively little is known at present concerning the nature of the antigen-antibody systems described in a variety of laboratories, it is clear that one or more antigens have been detected in the serum or liver of experimentally infected chimpanzees as well as in patients with acute and chronic non-A,non-B hepatitis. The specificity testing that has been possible with a limited technology suggests that although nonspecific antigens and antibodies are present during hepatitis, some of the antigens detected in serum are associated with non-A,non-B hepatitis. It is also clear that subsequent to or during non-A,non-B hepatitis, patients and chimpanzees often develop antibodies to these antigens. There may also be cross-reacting antibodies. Some antibodies, like those to hepatitis B core antigen, may indicate either recovery from or chronic infection with a non-A,non-B virus. Whether infectious virus persists in the serum in the presence of one or more of these antibodies is yet to be determined. One antigen detected in the sera of implicated blood donors, however, does coexist with infectious virus.

Four laboratories have reported the detection by immunofluorescence of a nuclear antigen in hepatocytes specific for non-A,non-B hepatitis. Two were in biopsies from experimentally infected chimpanzees, and two were in liver biopsies from human patients. The immunological specificity of this antigen-antibody system appeared to be good. Validation of this specific immunofluorescent system may help sort out the identity of some of the serum antigens by using each antigen to block the reaction between antibodies and the nuclear antigen. It remains to be determined whether any of these tests for antigens or antibodies can detect individuals who transmit non-A,non-B hepatitis.

Further work is needed to identify the agents of non-A,non-B hepatitis, to develop specific tests to detect carriers, to investigate virus inactivation (or neutralization by specific antibody), and to develop a vaccine for active immunization.

Before the development of sensitive serologic screening tests, measures designed to prevent hepatitis B included the permanent exclusion of individuals from donating blood who have a history of hepatitis, the temporary exclusion of those who either have received a blood transfusion or who have been in contact with a case of hepatitis in the recent past, and

the labeling of blood as "paid" or "volunteer" depending upon its source. It has also been shown that there is an increased risk of non-A,non-B hepatitis associated with the receipt of blood from paid donors. In one retrospective study, 128 individuals were evaluated 1 to 6 years after their blood had been implicated in the transmission of hepatitis to recipients. A minimum of 77 percent of these donors had transmitted non-A,non-B hepatitis according to serologic tests. None of the individuals had a history of hepatitis, and all were acceptable as blood donors at the time of the initial donation. Of these, 93 percent had never received a blood transfusion, and when studied retrospectively, 80 percent had normal serum aminotransferase levels. These individuals could not be differentiated from other donors on the basis of answers to additional historical questions. Non-A,non-B hepatitis after transfusion, therefore, remains a problem in the United States despite the interdiction of blood donors with a history of hepatitis.

A greater prevalence of serologic markers, consistent with either active or past hepatitis B infection, is detected among prospective donors with a history of hepatitis. A valid serological test for non-A,non-B hepatitis is not available so that screening for a history of hepatitis to identify donors who transmit non-A,non-B hepatitis is not yet possible. It should be noted, however, that HBV and the non-A,non-B hepatitis virus appear to be present among similar populations and that both are capable of producing chronic infections.

Chemical and Immunological Tests. Antigens and enzyme activities in serum have been proposed as methods to identify prospective blood donors who may have acute or chronic non-A,non-B hepatitis.

- Serum Aminotransferase Levels. Alanine aminotransferase activity (ALT) is usually elevated during acute hepatitis regardless of the etiology. ALT activity is often elevated in chronic hepatitis B, but of 128 individuals identified retrospectively as being chronic carriers of non-A,non-B hepatitis and tested for ALT elevations, only 20 percent had elevated ALT activity. The possibility does exist, however, that many more of these individuals had elevated ALT activity at the time that their blood transmitted non-A,non-B hepatitis. It has been shown that individuals chronically infected with non-A,non-B hepatitis can have normal ALT activity even when their sera transmit this form of hepatitis.

  In a study conducted by a transfusion-transmitted viruses study group, it was found that non-A,non-B hepatitis

occurred in 3.4 percent of recipients of units of blood with normal ALT activity compared to 38.9 percent of recipients of at least one unit of blood with elevated ALT. These data suggest a risk of non-A,non-B hepatitis associated with elevated ALT activity although the elevation has yet to be shown to be independent of other factors.

- Carcinoembryonic Antigen. The use of tests for carcinoembryonic antigen (CEA) to identify prospective blood donors at risk of transmitting hepatitis has been considered. Data from a study of donors have indicated a 46 percent incidence of hepatitis transmitted from donors with elevated CEA activity compared to 16 percent from donors with normal CEA activity. Also, in 18 of 29 cases of non-A,non-B hepatitis (62 percent), CEA activity during acute hepatitis was elevated. Since these findings have not been confirmed, the use of CEA activity as a screening test would appear unfounded at this point. In addition, elevated CEA activity has been reported in 11 percent of asymptomatic adults and in 5 percent of asymptomatic adolescents. Elevated CEA activity in apparently healthy, asymptomatic individuals might represent silent carriers of non-A,non-B hepatitis in some cases or, more likely, a lack of specificity associated with the test. The greater prevalence of elevated CEA activity among tobacco smokers who are apparently healthy suggests that because of a significant range of nonspecificity, this test should not be routinely used to screen blood donors.

- Alpha-Fetoprotein. No relation between serum alpha-fetoprotein (AFP) concentrations and non-A,non-B hepatitis has been described. In a group of Caucasians living in Greece, serum AFP concentrations were determined by radioimmunoassay in 80 patients with primary hepatocellular carcinoma (PHC), 40 with metastatic liver cancer (MLC), and 204 controls; PHC cases had significant AFP elevations when compared to those with MLC. Among controls, none had abnormal AFP concentrations. Chronic HBV carriers had significantly more AFP compared to noncarriers ($p < 0.01$). Whether AFP of patients with acute or chronic non-A,non-B hepatitis will be of use in diagnosing or managing such patients or in the detection of carriers needs to be determined.

- Serum Bile Acids. Concentrations of serum bile acids of chimpanzees in the fasting state were measured by radioimmunoassay in weekly serum samples during 17 experimental infections with HAV, HBV, and non-A,non-B hepatitis virus. Cholyglycine levels were elevated consistently during hepatitis A and hepatitis B but only minimally during

non-A,non-B hepatitis. The sulfated conjugates of lithocholate were elevated during hepatitis A and non-A,non-B hepatitis but not during hepatitis B. Elevations of sulfated conjugates of lithocholate in the presence of normal cholyglycine levels were detected only during non-A,non-B hepatitis. Whether sensitive assays for bile acids or whether the ratio of different individual bile acids can be used as a means to diagnose non-A,non-B hepatitis cases or to identify carriers of non-A,non-B virus is currently under investigation.

- Immune Complexes. Serial serum samples from 22 patients with transfusion-associated non-A,non-B hepatitis and from 2 chimpanzees with experimentally induced non-A,non-B hepatitis were tested for the presence of circulating immune complexes by Raji cell radioimmunoassay; 13 patients (59 percent) and 1 chimpanzee (50 percent) had detectable circulating immune complexes immediately before, simultaneous with, or during the return to normal of aminotransferase levels, and 7 of 10 patients (70 percent) with chronic non-A,non-B hepatitis had amounts of detectable immune complexes that varied directly with aminotransferase activity. It was suggested that these immune complexes could contain either viral antigens or viruses associated with non-A,non-B hepatitis.

  To confirm or extend these observations, other investigators examined serial serum samples from 15 patients with non-A,non-B hepatitis and 40 normal individuals for immune complexes by an anti-antibody inhibition test. The antibody used in this assay was an IgM immunoglobulin, which specifically combined with $F(ab)_2$ fragments of IgG that have undergone transformation as a result of reacting with antigen. Immune complexes were found in 1 of 40 normal sera (2.5 percent), 9 of 15 sera (60.0 percent) from acute hepatitis cases, and 5 of 10 sera (50.0 percent) from chronic hepatitis cases. Single-stranded DNA appeared to be the antigen in several cases. These observations suggest that the immune complexes associated with non-A,non-B hepatitis might represent autoimmune antibodies.

  Immune complexes have been noted to be 15 times more common in the sera of blood donors associated with hepatitis than in normal controls. In these studies, only 0.98 percent of "normal" blood had detectable immune complexes. Whether these individuals with circulating immune complexes transmit hepatitis needs to be studied.

- Frozen Deglycerolized Erythrocytes. The use in transfusion of deglycerolized red blood cells that have been frozen has been considered as a means to prevent the

transmission of viral hepatitis by blood. The failure of freezing and deglycerolizing red cells to remove HBV infectivity has been documented in one study of chimpanzees. Another is currently under way to determine the effect of freezing and deglycerolization of blood on the infectivity of non-A,non-B hepatitis virus. The relatively low titers of non-A,non-B hepatitis virus infectivity in sera of chronic carriers studied to date, as compared to the high titers common in sera of chronic HBV carriers, suggest that the use of frozen and deglycerolized erythrocytes theoretically may be more successful in preventing non-A,non-B hepatitis than in preventing hepatitis B.

Research Activities 1982 to 1987

- Develop in vitro tests to detect those who are chronically infected with HBV by increasing sensitivity or finding additional new markers.

- Investigate new uses for hepatitis B vaccine, such as in spouses of acute cases and children of HBV carriers.

- Investigate methods to detect those donors who transmit non-A,non-B hepatitis by:

  - Isolating and identifying particles, antibodies, antigens, and viruses

  - Developing in vitro tests to detect acutely and chronically infected donors

  - Determining the demography of those persons transmitting this infection.

- Investigate passive immunization against non-A,non-B hepatitis.

- Investigate physical, chemical, and immunological means to inactivate hepatitis viruses.

- Investigate the mechanisms by which individuals become chronically infected by hepatitis viruses.

- Investigate means to cure chronic carriers.

OTHER TRANSFUSION-TRANSMITTED DISEASES

State of Knowledge Through 1982

Diseases other than hepatitis that are transmitted by blood transfusion include bacteria, syphilis, malaria, nonmalarial parasites, cytomegalovirus, Epstein-Barr virus, and exotic viruses. Most important in this group is probably CMV, which causes obvious illness in immunosuppressed patients. Effective prevention and treatment have not been developed.

Several studies indicate that immunocompromised CMV-seronegative recipients of organ transplants or blood transfusions are at risk for significant complications of primary CMV infections. In such CMV-seronegative recipients, primary CMV infection is prevented by the selection of seronegative organ or blood donors or, possibly, frozen or leukocyte-depleted red blood cells. The epidemiology of posttransfusion CMV infections is most clearly delineated in newborn infants because reactivation of latent CMV is not a complicating factor, as it is in older patients. Premature infants receiving blood transfusions represent a special subpopulation of immunocompromised recipients. Multitransfused, preterm infants develop a sometimes fatal complex of symptoms characterized by respiratory deterioration, hepatosplenomegaly, gray pallor, and atypical lymphocytosis. The most likely source of infection is exposure to maternal cervical secretions at birth or, more probably, the multiple blood transfusions received by these infants in the course of treating their primary diseases. In view of the evidence that premature infants appear to be at risk for significant morbidity and mortality from transfusion-associated CMV infections, it is appropriate to consider selecting CMV-seronegative blood for transfusions in such infants.

Although posttransfusion CMV infection is not a major etiologic factor in the overall epidemiology of congenital cytomegalic inclusion disease (CID), the long-term consequences of CID are so severe that prevention of even the small number that is potentially related to transfusions would be a significant contribution. Currently, there are an estimated 40,000 cases of CID annually in the United States with 10 percent of infected newborns manifesting significant neurologic complications. While most cases of CID are presumed to result from reactivation of endogenous maternal CMV, primary maternal infections do occur and have a 50 percent rate of fetal infection.

Since pregnant females at risk for CMV-posttransfusion infections are readily identifiable by blood banks, it would be a desirable and reasonable goal to design programs to prevent transfusion-related CID by selecting CMV-seronegative blood for CMV-seronegative females.

Program Goals 1982 to 1987

- To confirm sources of CMV infections in transfusion recipients, evidence is needed directly linking donor and recipient strains of CMV. Such information is now potentially available through the use of analysis by restriction endonuclease digestion and the comparison of migration patterns of DNA fragments from donors and recipients. These new methods should permit additional epidemiologic data.

- It is important to determine whether otherwise benign posttransfusion CMV infections may pose a hazard to others, such as other newborns, mothers, and pregnant nurses in the nursery.

- Knowledge is needed of the extent to which posttransfusion CMV infections in premature infants are a complication of replacing blood sampled for intensive care monitoring. Knowledge is also needed of whether further miniaturization of clinical laboratory test equipment would offer a complementary, if not alternative, approach to reducing posttransfusion CMV infections in newborn infants.

- In view of reports that premature infants of CMV-seronegative mothers are at risk for morbidity and mortality from posttransfusion CMV infections, it is appropriate to consider providing CMV-seronegative blood for selective transfusions in these infants. No consideration, at present, is being given to supplying "CMV-free" blood products to pregnant females or to immunosuppressed patients, including transplant recipients. If such programs are instituted, studies should be made to evaluate the impact of these changes on previous or current incidence of posttransfusion CMV infection.

Research Activities 1982 to 1987

- Areas for research should include vaccine development and testing, development of new antiviral chemotherapy that might be active against CMV, and the development of sensitive screening methods that are economically feasible for large-scale screening of donors.

Apheresis

The term "apheresis" is derived from the Greek word "aphairesis" (literally, taking off, from aphairen--to take away, from

apo + hairen--to take, with the noun-ending sis). The word is used to describe the removal of any portion of the vascular fluid, but not usually the therapeutic removal of whole blood. By use of closed-system centrifuges, blood is removed from the donor, the desired component is separated, and without any interruption in the procedure, the remaining portion is transfused back into the donor. The following terms have been compounded with "apheresis":

plasmapheresis: removal of plasma for fractionation into derivatives. Plasma obtained by apheresis for the purpose of preparation of derivatives is known as "source plasma," as distinguished from "recovered plasma," which is separated from whole blood collections.

plasma exchange: removal of plasma for therapeutic purposes, usually accompanied by return of electrolyte solutions or albumin-containing solutions.

leukocytapheresis (leukapheresis): removal of white cells.

lymphocytapheresis: removal of lymphocytes.

thrombocytapheresis (plateletapheresis): removal of platelets.

In the 1972 National Program, no mention was made of apheresis, although in its Report of 1976, the Blood Diseases and Resources Advisory Committee commented on the need for automated cell-separating devices for isolating platelets and granulocytes in order to provide single-donor concentrates of these cells. The Fifth and Seventh Reports of the Director, NHLBI, made reference to improved technology for apheresis and noted the growing use of therapeutic apheresis in the treatment of a growing number of conditions.

Progress and State of Knowledge Through 1982

During the 1940's, the exigencies of World War II led to an increasing demand for human plasma. Initially, plasma was obtained by taking a unit of whole blood from a donor, separating the erythrocytes from the plasma, and using each separately for transfusion. In many settings, however, the demand for plasma exceeded that for erythrocytes, and it was apparent that the factor that limited the frequency at which blood could be taken from a human donor was the rate of regeneration of the red blood cells and hemoglobin. The U.S. Public Health Service reported that in 1943, 5 million units of plasma were required by the U.S. armed forces and that most of the red cells were discarded;

375,000 kg of human hemoglobin were wasted. Several groups met this problem by introducing to humans the technique of plasmapheresis, which had first been employed on animals in 1914.

During the next 20 years, the requirements for products obtained from the fractionation of human plasma expanded rapidly. In 1968, the Committee on Transfusion and Transplantation of the American Medical Association conducted a survey in which some 200 establishments were identified as conducting or "presumably conducting" plasmapheresis programs. The establishments used 90,000 donors and generated 400,000 liters of plasma annually. By 1979, the annual production had risen to 4 million liters of plasma, and industry projections suggested that in 1981 some 6 million liters would be produced by plasmapheresis. The Collection, Fractionation, Quality Control, and Uses of Blood and Blood Products, published by the World Health Organization (1981), lists no less than 17 medically important substances derived from human plasma.

The equipment for separating blood cells from plasma was simple until the mid-1960's. Blood was removed from the patient into an anticoagulant solution, usually in a plastic container, which was subsequently disconnected and centrifuged to separate the cells. The plasma could then be transferred to a second plastic bag, while the red cells were returned to the donor. In recent years, selective apheresis has been employed to remove individual components (cells or plasma) from the blood of normal individuals, to use for transfusion. More recently there has been a rapid expansion of interest in the removal of plasma or blood cellular elements as a means of treating diverse diseases.

The first successful separator that could salvage cellular components and return unwanted portions to a donor was constructed at the NIH in a joint project with IBM in the early and mid-1960's. The result of that collaborative effort was the NCI-IBM cell separator, a continuous flow centrifuge in which the fixed parts for inflow and outflow of blood were joined to the rotating portion by a lubricated ceramic rotating seal that remained cool and did not generate shear stress, which could cause hemolysis. The major goal of the developers of these first cell separators was to collect normal human granulocytes in large enough numbers for transfusion. The device was also found suitable for platelet collection and has more recently been applied to the isolation of young red cells ("neocytes"). It was also capable of performing plasma exchange in a short time on a scale that by previous standards was relatively massive. Experience with this device, therefore, laid the groundwork for the enormous number of applications for blood separator machines that has occurred in recent years. Several other models of blood cell separators have been developed and are now in clinical use.

Important technical improvements include the increase in separating capacity, the development of disposable tubing through which the blood passes, greater sophistication in preventing and identifying failure in the system, automatic alarm systems for warning of failures of flow and accumulation of air, and an increasing degree of automation of control functions. Elimination of the rotating seal disposed of one common source of unreliability and brought about a decline in the risk of contamination.

Recently, a different approach to phasmapheresis has been developed. This technology depends on membrane filtration that uses biologically inert semipermeable materials, which allows rapid transfer of selected compounds from the plasma. While earlier equipment was relatively slow, had a tendency to activate complement, and induced some changes in cellular characteristics, newer membranes may be less reactive and more selective, and allow more rapid separation of plasma. When used in tandem, membranes with different molecular characteristics remove substances within a narrow range of molecular sizes, in effect cleansing the plasma and allowing the purified plasma to return to the patient. This procedure eliminates the need for extensive replacement of lost fluid and the waste of physiologic substances.

The latest major improvement in instrumentation consists of hollow fiber membrane filtration units, which are being investigated actively in Europe and Japan, where extensive experience has already accumulated in their use as a therapeutic modality in many hematological, renal, and neurological disorders, many of which involve immune complex formation.

Plasmapheresis, combined with low-temperature exposure to remove cryoproteins or with sequential passage of plasma through various absorbant columns, further extends the range of therapeutic options for apheresis and permits the consideration of many disorders, including toxic and malignant conditions, for such therapy.

It is difficult to estimate accurately the number of apheresis procedures being performed in the United States either for collection of components for transfusion or for therapeutic purposes. The American Red Cross has reported the following:

- Fiscal Year 1979 to 1980

Total, all apheresis procedures	44,000
Therapeutic apheresis	1,750

- Fiscal Year 1980 to 1981

Total, all apheresis procedures	46,000
Therapeutic apheresis	2,900

The Canadian Plasma Exchange Group has documented that in 1980 to 1981, with a population of 25 million, plasmapheresis was performed in Canada on 343 patients, to a total of 3,973 procedures. The American Blood Resources Association estimated that 5.5 to 6 million liters of plasma to be used for the preparation of blood products would be removed from donors by plasmapheresis in 1981. By comparison, it has been estimated that approximately 60,000 therapeutic plasma exchange procedures would be performed in 1981. The majority of these represent plasmapheresis or combined plasma and lymphocytapheresis, though some are performed for the removal of excess numbers of circulating blood components.

Therapeutic Apheresis

A natural sequence to the development of efficient automated systems for the removal of plasma or specific cellular elements from normal donors was the effort to reduce excessive concentrations of plasma proteins or cellular blood elements.

Some of the earliest and most dramatic results were obtained in patients with paraproteinemias who were suffering from peripheral vascular or cerebrovascular complications of increased blood viscosity. Exchanging large amounts of their hyperproteinemic plasma with electrolytic solutions or with mixtures of electrolytic solutions and serum albumin often led to rapid clinical improvement.

Rapid removal of leukemic cells or of excessive numbers of platelets has also been associated with clinical improvement, albeit with less consistency than is obtained with plasma exchange in hyperproteinemic patients.

The most exciting and controversial uses of therapeutic plasma exchange have been in the treatment of a variety of disorders of presumed immunologic etiology. While some remissions have been induced in systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, and Goodpasture's syndrome, and quite frequent improvement is seen after plasma exchange in myasthenia gravis, the specific indications and preferred techniques remain to be clarified. The role of concommitant forms of therapy, such as the immunosuppresants and plasma replacement, must also be ascertained.

Although it is generally agreed that plasma exchange is a reasonably safe procedure, it is essential that its hazards be documented and discussed. A number of centers have described the occurrence of life-threatening infections following plasmapheresis. Deaths have been encountered in two patients due to sudden widespread occlusion of the pulmonary vascular bed. Both

received fresh plasma as replacement fluid. At least three other deaths due to cardiac arrhythmia have also been reported.

The incidence of morbidity and mortality associated with plasmapheresis would not appear unduly high if the procedure were limited to patients with severe and potentially fatal disease and if the benefits of the procedure were clearly and unequivocally documented. However, the possibility of hazardous reactions must be taken into account in planning trials, along with calculations of the cost efficiency of plasma exchange procedures.

The cost of therapeutic plasma exchange is made up of the cost of replacing albumin (approximately $120 per liter, with an average exchange of 2 liters) and the charge per procedure for equipment, disposable supplies, and professional services (approximately $100). With an average cost per procedure of $340, the total cost for 60,000 therapeutic plasmaphereses exceeds $20 million. The cost when only lymphocytes are removed is less, because no expensive replacement fluids are required. It is estimated that the total number of therapeutic plasmaphereses will reach 80,000 in 1982 and 100,000 in 1983. Assuming that costs remain stable, the expense of therapeutic apheresis may rise to $34 million in 1983. If new applications of therapeutic plasmapheresis emerge, there could be a substantial increase in the demand for this service.

Program Goals 1982 to 1987

Therapeutic plasmapheresis and therapeutic cytapheresis (especially lymphocytapheresis) have been available as techniques for a number of years. Within the last decade, however, the availability of discontinuous and continuous centrifugation devices have made possible the separation of large volumes of cells and plasma from diseased subjects. The application of these devices has dramatically increased interest in therapeutic apheresis. The number of such procedures has risen steadily. If plasmapheresis and lymphocytapheresis prove to be effective in controlling rheumatoid arthritis and other disorders, this therapeutic modality represents an important step in the management of disabling conditions, and the likely extent of the demand for this approach will represent a major financial problem.

Because of the important economic repercussions that will follow if therapeutic apheresis is found to be important in the management of diseases as common as rheumatoid arthritis and multiple sclerosis, energetic efforts should be devoted to adequately regulated and well-controlled studies designed to demonstrate the possible efficacy of these new treatments. It is also important to determine if any economic benefits can be expected to flow from their widespread application. Until

recently, a number of centers were applying plasma exchange and lymphocytapheresis in an uncontrolled manner in the treatment of patients with rheumatoid disease. Until such treatments are proven to be efficacious, the situation is unsatisfactory. On the recommendation of the National Center for Health Care Technology, third-party payers determined in 1981 that the indications for plasmapheresis in rheumatoid arthritis should be limited to certain defined complications such as vasculitis or hyperviscosity. There is considerable indirect evidence to suggest that the volume of plasmapheresis operations performed for rheumatoid arthritis has fallen substantially as a result of this decision.

In parallel with applied research and clinical trials in therapeutic plasmapheresis, it is important to study physiologic changes created by the violent perturbation of plasma exchange. Greater long-term benefit may be expected from an understanding of the fundamental mechanisms of disease than from the simple assessment of the efficacy of different methods of treatment.

Since plasma exchange and cytapheresis are now major components of therapy for a number of diseases and since the procedure is not without risk, encouragement should be given to the rapid sharing of any new information in scientific meetings and through publications.

Research Activities 1982 to 1987

Present equipment for therapeutic plasmapheresis and cytapheresis is costly and cumbersome. Simpler equipment capable of performing the same procedures with greater safety is desirable.

New applications should be explored for therapeutic apheresis. Innovative investigators should be encouraged to apply therapeutic apheresis to small groups of patients and to describe the results objectively. Once a promising line of investigation has been established, there is at present an unacceptably long delay before it is possible to initiate controlled trials. Everything possible should be done to move swiftly at this stage so that an objective evaluation of the usefulness of therapeutic apheresis can be quickly determined.

When clinical studies indicate that therapeutic apheresis is beneficial, it should be made available promptly and safely, and with minimum financial hardship to those patients with diseases that have been clearly shown to benefit from this procedure.

Specific Recommendations

- Foster clinical or basic science studies to clarify the value (if any) of plasma exchange in various diseases.
- Foster accumulation of data detailing the extent of apheresis activities in the United States for component collection and for treatment.
- Encourage development of rapid, inexpensive systems for collection of plasma for fractionation so that volunteer donors might be recruited for this purpose.
- Support investigation and development of methods for selectively removing materials from plasma that are associated with clinical conditions.

Blood Resources Management

According to the 1972 National Program:

> Managing the national blood resource requires a combination of human resources, sociologic, economic, scientific, medical and management expertise and technology, instrumentation and manufacturing capabilities.

Recognizing that the blood services complex has not consistently maintained an adequate supply of blood or achieved the highest attainable quality of blood therapy, framers of the program went on to state:

> The goal of this program is to achieve an adequate supply of high quality blood components by utilizing the National blood resource with maximal efficiency, economy and safety.

In 1973, the Secretary of Health, Education, and Welfare issued the National Blood Policy, which called upon the blood banking community and other interested persons and agencies to cooperate with the government in achieving four goals for blood resoures: an adequate supply, a high quality, accessibility (affordability) to everyone, and improved efficiency in collection and delivery. An objective was to establish an all-volunteer blood-donor system and to eliminate commercialism.

The National Blood Policy was to achieve the coordination of private and public organizations in an all-volunteer blood-donor system through regional integration and cooperation of blood-distributing activity. Resource sharing, which was a program to

reduce waste and improve utilization by transferring blood between regions, was to be a prominent feature of this effort. Blood data and information, obtained through a national center, were to be used in evaluating the system and designing necessary changes. The quality and effectiveness of the nation's blood supply was to be upgraded through research and development on automated inventory control methods, techniques for projecting blood needs, ways of increasing donor motivation, and approaches to educating the physician in optimal use of blood.

Major interest in the Natonal Blood Policy dominated the first five Reports of the Director, NHLBI. It was noted in these annual summaries that the Institute was cooperating in the establishment of the National Blood Policy, was initiating a Blood Data Collection Center, and was aiding the process of regionalization.* Cost-finding methodologies, recruitment programs, and related items were also pursued. A great deal of attention was devoted to the development of educational programs in blood and component use for health care professionals with direct concern.

The advisory committee report of 1976 also focused on these considerations and highlighted the function of the American Blood Commission (ABC), which was organized in 1974-1975 in response to the Department's call for a National Blood Policy. A consortium of medical, labor, and consumer organizations, the ABC was formed to implement the goals of the National Blood Policy and to serve as the single locus of accountability to the government. Reports on the supply and use of blood and on personnel needs in blood banking were developed by contract with the ABC. The advisory committee recommended that the DBDR continue its support of the National Blood Policy by assisting the ABC in achieving the following goals: an all-volunteer blood-donor system, interregional cooperation, a national data collection system, a blood management and distribution system, blood registries, development of equipment, and training and education in the proper use of blood and its components.

Progress and State of Knowledge Through 1982

Since 1971, the percentage of volunteer donors has risen from about 85 percent to an estimated 98 percent. As might be expected, the number of commercial blood banks has been drastically reduced in every part of the country. In addition to

*The formation, within stated geographical areas, of associations of blood service units in which blood providers and blood users cooperate in the administration of blood resources, following criteria developed by the American Blood Commission.

increasing voluntarism, the ABC promoted more uniform third-party payer policies respecting reimbursement of blood services.

Regionalization has proceeded slowly but steadily. According to the ABC, approximately 20 percent of the country is now covered by regions recognized by the ABC as meeting its criteria. These regions account for 20 percent of the national blood supply. Other regions are in various stages of recognition.

Although the decade of the 1970's was marked by serious disagreements among the three national blood banking organizations (American Association of Blood Banks, American Red Cross, Council of Community Blood Centers), interregional blood transfers between affiliates of the groups were effected through a variety of mechanisms. Most of the transfers, amounting to about 7 percent of the total national blood supply, were arranged on an ad hoc basis. After alleged shortages were brought to the attention of Congress in 1979, the three parties, with the assistance of the ABC, began resolving their differences. Starting in mid-1982, resource sharing is to be administered by an autonomous group unaffiliated directly with any of the four participating organizations but with oversight of a board comprised of representatives of each. In addition to meeting spot needs, the program will undertake long-range planning to promote the efficient transfer of resources from regions of oversupply to those unable to meet demands fully.

At the present time, estimates place the volume of blood shipped between regions at about 5 percent. Some of this improvement over earlier estimates is undoubtedly attributable to the use of CPDA-1, a new preservative solution approved for use by the FDA in 1979, which permits the extension of the shelf-life of whole blood and red blood cells from 21 to 35 days. CPDA-1 has reduced outdating, improved utilization rates, and reduced dependence upon other regions.

The combined efforts of the NHLBI, FDA, ABC, and the major blood banking organizations also facilitated the compilation of a useful and comprehensive data base of blood banking activities in 1979 and 1980. These are the first valid figures since the NHLI survey of 1971-1972.

Much of the impetus for a National Blood Policy arose from the unacceptably high rate of posttransfusion hepatitis associated with the use of commercial blood. The introduction and mandatory use of techniques for detecting and excluding hepatitis B carriers coupled with the decline in the use of commercial blood have improved the quality of the blood resource by reducing the incidence of hepatitis B transmitted by transfusion from an estimated 30 to 50 percent to only about 10 percent of current cases. The majority of the remaining cases represent another disease process

(termed non-A,non-B hepatitis) produced by another etiologic agent.

In very great part, the 1972 National Program was the basis for the National Blood Policy. With respect to blood resources, the Institute gave up much of its autonomy when it funded the unsolicited contracts of the ABC. Thus, when the policy addressed a "partnership" between the public and private sector, one of the major bonds of that cooperative relationship was the financial stewardship of the Institute in spreading the philosophy of voluntarism and in improving efficiency through regionalization and resource sharing. The partnership also successfully preserved the pluralism of the American blood bank system while strengthening the linkages between its components.

Although voluntarism is generally embraced by all of the ABC members and the government, it was not universally endorsed in 1972. Many economists, including some within the government, suspected that voluntarism might lead to harmful cartelization, inhibit competition, and foster ever increasing costs and charges. The precept, however, is now so widespread that it is unlikely to be shaken by a return to commercialism or by changes inspired by the competitive market forces now operating within the health care community.

The National Blood Data Center (NBDC), which was called for in the 1972 Program and was created by the ABC, was shown to be too ambitious and costly to survive as an independent data-gathering and analytic body. The NBDC did create a successful framework for the continued evaluation of the blood supply and of costs and utilization that could operate as an integrated function of another activity such as resource sharing.

Almost coincident with the 1972 National Program, the Bureau of Biologics was transferred from the NIH to the FDA, where its regulatory authority was greatly enhanced. Under the FDA, standards of blood banking were extended to cover all blood collection and transfusion facilities. This action fostered the rapid introduction and use of technologies to detect hepatitis. In addition, the promulgation of rules requiring a "paid donor" label was effective both as an educational tool and as a sign to the commercial industry of the undesirability of its product. The totality of FDA action constituted a major contribution to the improvement of blood quality.

Program Goals 1982 to 1987

Opportunities obviously exist in technical areas, such as the development of improved anticoagulants to increase the shelf-life

of blood and its components and the development of improved methods for preventing posttransfusion hepatitis.

There is also promise in the investigation of further uses of blood and blood components. The NHLBI-funded studies of the use of whole blood and red blood cells not only need to be extended to components such as platelets, but more importantly, translated into recommendations to reduce the unnecessary use of blood. Additional information is also needed about the impact of autologous infusion, especially of intraoperative techniques.

Research Activities 1982 to 1987

The original blood resources goals of the 1972 National Program and of the National Blood Policy have either been reached or are in progress. The remaining initiatives include further implementation of private-sector regionalization. In particular, regionalization needs to be extended to those areas in which there is not a cohesive program to assure reasonable noncompetitive collection and distribution practices. The data-gathering function begun by the National Blood Data Center must also be preserved. It is hoped that a national system of resource sharing will be equipped to incorporate data collection into the information system required for its long-term planning function.

No further legislative or regulatory action is recommended with respect to the issues addressed by the National Blood Policy and featured in the 1972 National Program. If the data collection funded by the NHLBI is incorporated into a national system of resource sharing, the NHLBI should consider obtaining data from this source to discharge its statutory obligations.

6. Prevention

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6. Prevention

Good health at a reasonable cost is a goal of our society, and the prevention of disease is an ideal approach to achieving it. Prevention requires:

- scientific knowledge for dealing with disease problems,
- demonstration, in an appropriate setting, of a new means of effective medical intervention,
- participation of adequately informed health professionals in the treatment and management of disease, and
- active interest of the public in achieving and maintaining its own health.

As one of the world's foremost biomedical research centers, the National Institutes of Health (NIH) has a long history of supporting and conducting research related to disease prevention, and important aspects of each of these four requirements are within its purview. The mission of the NIH is usually expressed in terms of the development of new knowledge that will lead to better health for everyone. The same idea also can be expressed as the development of new knowledge that will lead to the prevention of disease.

Basic and clinical research are traditionally associated with the primary activities of the NIH. Demonstration of the effectiveness of intervention procedures is also one of its responsibilities, as is the introduction of intervention procedures into the practice of medicine. When a new technological development has been shown to be effective in an appropriate medical setting, it is important that the community of health professionals be informed about it. The NIH achieves this "technology transfer" generally by distributing publications and by sponsoring seminars, workshops, conferences, and consensus development meetings. The NIH sponsors a few formal programs in classic "continuing education," which is the next step in the succession of medical progress, but the related area of postgraduate medical education that leads to the practice of medicine is not part of the legislated mandate of the NIH.

Public education, which is the term used by the NIH to refer to all aspects of the process by which the public is educated to

understand and appreciate its part in maintaining good health, has historically been only peripherally related to the range of NIH responsibilities. The Congress now appears to want the NIH to devote more attention to public education.

One possible reason for the interest in public education shown by U.S. citizens and, as a consequence, by the Congress is the recognition that optimal ratio of cost to benefit in the health care system is achieved by preventing the development of disease and its complications. If the public is to assume an important share of the responsibility for its own care, it must have the necessary information, understand its implications, and receive help in modifying its behavior in healthfully beneficial ways.

The uncertainties about this congressional intent and about the responsibility of the NIH in meeting it has continued through the last decade. Within the Division of Blood Diseases and Resources of the National Heart, Lung, and Blood Institute, the term "prevention, education, and control" has been considered to be an activity that has as its ultimate goal the prevention of disease or disability through various forms of medical intervention. Such intervention may be primary--it prevents the onset of disease, or secondary--it prevents the progression and complications of disease and minimizes suffering when a disease process cannot be interrupted.

The dissemination of information about approaches to medical intervention is a responsibility of health care professionals and of the public itself. Information is transmitted to professional groups through established mechanisms of education, but the need for more effective and less expensive educational methods remains. Similarly, information is presented to the public through a number of established media, but, again, major opportunities and needs for improving communications are apparent. An ultimate goal of public education is to inform people and motivate them to be responsible for their own health.

Through the combined efforts of health care professionals and an interested public, and through support of all aspects of prevention, the American people can improve their health and thus moderate the burden of health care costs. The decreasing rate of mortality from heart disease and stroke are dramatic examples of the success of such an approach. Critical to such success, however, is the continued support of scientific investigation as the first priority and as the basis of all prevention programs.

Terminology, Definitions, and Concepts

Most of the formal programs of the NHLBI in prevention, education, and control had their origin in the National Heart, Blood Vessel, Lung, and Blood Act of 1972 (P.L. 92-423):

> Section 413(d). There shall be in the Institute an Assistant Director for Health Information Programs who shall be appointed by the Director of the Institute. The Director of the Institute, acting through the Assistant Director for Health Information Programs, shall conduct a program to provide the public and the health professions with health information with regard to cardiovascular and pulmonary diseases. In the conduct of such program, special emphasis shall be placed upon dissemination of information regarding diet, exercise, stress, hypertension, cigarette smoking, weight control, and other factors affecting the prevention of arteriosclerosis and other cardiovascular diseases and of pulmonary diseases.
>
> Section 414(a). The Director of the Institute, under policies established by the Director of the National Institutes of Health and after consultation with the Council, shall establish programs as necessary for cooperation with other Federal Health agencies, State, local, and regional public health agencies, and nonprofit private health agencies in the diagnosis, prevention, and treatment (including the provision of emergency medical services) of heart, blood vessel, lung, and blood diseases, appropriately emphasizing the prevention, diagnosis, and treatment of such diseases of children.

Although Section 414(b) authorized $25 million, $35 million, and $45 million for the next 3 years, no funds were ever appropriated. As directed, however, the Institute responded by appointing an assistant director for health information programs and by creating an Office of Prevention, Control, and Education. Disease prevention and control programs eventually came to be called Prevention, Education, and Control (PEC) Programs, the function of which was presumably to highlight the basic mission of the NIH--to support research that would lead to the prevention, intervention (treatment), and control of diseases and abnormalities.

The DBDR has relied on slight modifications of the definitions of primary, secondary, and tertiary prevention proposed by the Ad Hoc NIH Working Group on the Definition of Disease

Prevention, which was chaired by Dr. Arthur Upton, then Director of the National Cancer Institute, in its report of August 4, 1978:

> Primary Prevention: intervention before the biologic onset of the disease in question. . . . Included is basic research indirectly relating to etiology and development of preventive measures, as well as direct preventive measures themselves, such as vaccination programs, regulatory activities, and public education. Included also are epidemiologic studies allowing for the identification and quantification of risk factors relating to personal characteristics such as habits associated with diet, use of cigarettes, alcohol consumption, exercise and the like.
>
> Secondary Prevention: intervention when disease can be detected but at a step before it is symptomatic, such as screening of premature infants for hyaline membrane disease before onset of symptoms, treatment of asymptomatic hypertension to prevent cardiovascular complications, myocardial infarction, and stroke. This may include basic research related to diagnosis, development of diagnostic methods, and diagnostic intervention, such as screening programs for the detection of disease.
>
> Tertiary Prevention: alleviation of disability resulting from disease after symptoms have already become detectable, in an attempt to minimize ensuing morbidity and mortality, and to restore normal functioning, such as anticoagulant therapy after thrombosis and rehabilitation after a stroke.

The DBDR has used the working group's definition of primary and secondary prevention as a basis for a concept of "prevention" that is separate from the concept of "control." In this usage, "prevention" refers to intervention before the biologic onset of a disease, which includes direct preventive measures such as vaccination programs and basic research that may be indirectly or inapparently related to etiology and to the development of preventive measures. (Also included are epidemiologic studies that allow for the identification and quantification of risk factors related to personal characteristics and habits.) The DBDR also operationally includes intervention efforts to detect a disease at a step before it is symptomatic, such as basic research related to diagnosis, development of diagnostic methods, and screening programs (diagnostic intervention). The working group's definition of tertiary prevention has provided a basis for a suitable definition of the term "control" as related to alleviation of disability resulting from disease after symptoms become detectable: attempts to minimize ensuing morbidity and mortality and to restore normal functioning, such as in anticoagulant therapy after thrombosis.

The Division's working definition of prevention is essentially consistent with that found in the 1972 National Program:

> Prevention of heart and blood vessel, lung, and blood diseases will require not only the acquisition of new knowledge through research programs but also the demonstration to the medical profession and the public of the applicability of this knowledge. Further, prevention will require local community and regional participation in the control of these diseases.
>
> [The consultants believed] that demonstration and control programs [would] help to effect the transmission of fundamental research advances to the public and thereby help to promote the health of our citizens. These programs are an essential link between biomedical research and health care. (DHEW Publ. No. [NIH] 73-515)

In the recently issued NIH report on Research in Prevention, Fiscal Year 1980 [no publication number], the NIH discussed only those aspects of prevention that are related to intervention, before the biologic onset of disease, with direct preventive measures. Methods of "secondary prevention"--intervention when disease can be detected before it is symptomatic--were included only if they helped prevent degeneration after the onset of symptoms. Of the ten areas listed for the NHLBI, two are the responsibility of the DBDR: education and counseling programs for sickle cell disease and prevention of deep venous thrombosis. Although the Division does support education and counseling efforts in sickle cell disease, its prevention efforts are far more extensive than what is reflected by the fiscal statistics given in the report. In fact, the entire sickle cell disease program is an example of a prevention, intervention, and control program in the area of a single disease.

The concept that prevention, education, and control are primarily an information-dissemination activity is a relatively new one, and the responsibility of the NIH to sponsor programs in these areas has caused concern among many. According to the Report of the President's Biomedical Research Panel (April 30, 1976):

> The primary mission of the NIH, as constituted today, is fostering, supporting, and conducting laboratory and clinical research to increase our understanding of life processes and the etiology, treatment, and prevention of diseases. The Panel has found no body of opinion urging a change in this primary function of the NIH. Many individuals, however, have pointed out that fulfillment of this role has become more difficult. The difficulty appears to arise from two evolving conditions: growing

> competition for resources between the research mission of the NIH and the application and dissemination of knowledge, and increasing and frequently changing public demand for allocation of resources according to public perceptions of important health goals, rather than on the basis of scientific opportunities. . . .
>
> A large portion of the research supported by the NIH is fundamental research in biomedical science that will provide the science base upon which to build improved technologies for the prevention and cure of diseases. Before there can be intelligently conducted applied research, before there can be demonstrations of clinical trials, or before there can be public dissemination of techniques, there must be enough productive basic research to provide the fundamental science from which these efforts flow. (DHEW Publ. No. [OS] 76-500)

In discussing the specific topic of the "Application of Dissemination of Knowledge," the panel revealed an ambivalence about the extent to which the NIH should engage in "transfer functions"--by then, a term frequently used to describe PEC activities:

> The development of new knowledge is the basic mission of the NIH. In addition to its basic mission, the NIH must explore applications of new knowledge that are effective in health care and must assist in disseminating this new knowledge to appropriate groups. The degree to which the NIH engages in these "transfer" functions and the problem of resource allocation for these activities as distinct from basic research functions has raised troublesome and complex issues in the science community, in the NIH, in the DHEW, and in the Congress.
>
> The congressional authorizations in 1971 and 1972 for high-priority programs in cancer and heart disease greatly expanded the scope of the NIH in the fields of knowledge application and dissemination and moved it closer to conducting clinical service programs. This has led to differences of opinion regarding the proper role of the NIH. Many in the science community prefer that the NIH revert to a "pure" research institution. Others within this same community and elsewhere feel that this new responsibility is appropriate and that the mission of the NIH encompasses knowledge applications in the interest of improving health care and public well-being.

Although the panel concluded that

> the primary role of the NIH should continue to be that of conducting and supporting laboratory and clinical research attuned to the search for new knowledge and, given adequate resources, of conducting and supporting clinical trials, selected demonstrations, and selected educational programs,

the panel, nevertheless, recommended that

> each Institute of the NIH . . . should organize a formal structure for knowledge application and dissemination activities. Each must provide leadership in this effort to assure that the latest scientific findings bearing on health care are made available to the professional community. While the NIH . . . cannot take responsibility for providing large-scale programs for physician education, [it] can . . . cooperate with the private sector in disseminating knowledge of new clinical advances.

The panel's main concern, which was related to its priorities for the NIH, was expressed in the final sentence of the recommendation:

> Knowledge application and dissemination activities and clinical trials should be staffed and funded by resources dedicated solely to these purposes and should not compete with research budgets.

The President's Biomedical Research Panel was so concerned about "the transfer of scientific innovations" that it sponsored two studies of the sequence by which a laboratory discovery moves to widespread clinical application. Both studies were retrospective examinations of the history of medical advances: one was an inquiry into the processes by which 132 advances in cardiovascular and pulmonary medicine and surgery came about, and the other consisted of 25 case histories of successful innovations over a period of 40 years, with a focus on the characteristics of the critical events in the development of the innovations. One of the most important conclusions of these studies was that many of the factors that influence progress or that cause delay are not amenable to action by the Federal Government; rather, these factors involve scientific questions that can be answered only by the scientific community.

Other conclusions supported the perception that planned intervention can accelerate movement from discovery to application. For example, in the study of the 25 cases of innovation, there was no evidence of an inappropriate delay, or of a delay that was amenable to intervention, between the completion of a

development and its general availability. Frequently, there was a substantial interval between the first clinical application of a discovery and its widespread use by practicing physicians and, indeed, its acceptance by patients, but the length of the interval was beyond the control of the research community. The principal cause for delay was the absence of a sound science base that would permit advancement. For the innovations that were introduced before 1951, the science base generally developed as a result of organized, or "targeted," programs. After 1951, such organized programs existed for only two of the remaining 16 innovations studied. This finding was considered to indicate that even with the accelerating pace of science, organized programs are not necessary for introducing important innovations into clinical practice.

While recognizing that the conclusions were derived from a very limited sample chosen from advances already in clinical use, the authors of these studies believed that

> the conclusions do not support the generalization that special action can never accelerate progress, but they surely help dispel the general myth of "undue delay" in making the benefits of science available to the health care of the nation. The Panel has developed a different concern during its deliberations: the fear that laboratory discoveries may be prematurely applied to health care in some instances where clinical validation has not occurred or has been inadequate. Such premature acceptance can pose just as serious a threat to the nation's health as any real delay in making new and proven technologies available.

It is apparent that the panel was apprehensive about intrusion into the process that incorporates useful knowledge in the prevention and control of disease. Moreover, the panel's concern about a shift of fiscal resources from support of research to support of demonstration and education activities is now a more critical issue than at any other time in the history of the NIH.

Also in April 1976, the Blood Diseases and Resources Advisory Committee submitted its Report to the Director, DBDR, which concluded with a discussion of "prevention, control, and education." The most significant discussion of this aspect of the report was in "Other (Potential) Applications." The committee's concerns here directly paralleled those of the President's Biomedical Research Panel:

> . . . We consider it important that clear definitions of such terms as "demonstration," "prevention," "control," and "education" be arrived at so that their relationship

> to ongoing basic and clinical research and particularly to clinical trials can be delineated. . . .
>
> Since there are effective mechanisms for the dissemination of knowledge to the deliverers of health care (such as publication in local, state, or national journals; staff and departmental meetings; graduate and postgraduate courses; seminars and medical society meetings; and specialty organization meetings . . . designed to provide information on new techniques), care must be taken that government-sponsored plans to facilitate the translation of knowledge from research into practice complement the existing mechanisms rather than duplicate or disrupt them. (DHEW Publ. No. [NIH] 76-1174)

The advisory committee chose not to offer recommendations for demonstration projects in areas of blood diseases and resources other than the two in which there were already "activities"--sickle cell disease and blood resources, even though they readily recognized that certain

> . . . areas might lend themselves to prevention, control, and education: management in blood banking, hemophilia care, screening of newborns for heritable blood disorders, and the introduction of iron supplements into the diet of the population at large. Information from clinical studies related to the diagnosis and treatment of deep-vein thrombosis, natural history of sickle cell disease, and treatment of aplastic anemia, as they reach the proper stage of development, may then require demonstration and educational efforts.

The advisory committee went on to recommend that

> . . . specific proposals dealing with PCE [prevention, control, and education] projects be subjected to peer review using the mechanisms similar to study section review or technical merit review. There is a need for developing definitions and descriptions of demonstration projects which should include in-depth documentation of need; description of test environment; documentation of expertise; specifics of demonstration objectives; [and] methods and evaluation instruments. Pending implementation of these recommendations, we defer identifying specific areas currently in need of demonstration projects in the field of blood.

The hesitancy of the scientific community to make more detailed recommendations, as represented by the observations shared by these two differently composed advisory groups, remains apparent today. Basic and clinical scientists wish to apply to

"demonstration" and "education" activities the same standard of excellence that they apply to research, but they are not certain of the expertise needed for it, and they are reluctant to endorse the commitment of fiscal resources to those areas as resources for biomedical research become more limited.

Effective Communication with the Public

Prevention of the onset of disease, with its obvious benefits to physical and mental health, is a practical means of controlling the cost of health care. The need for the public to develop a better perception of health and to make better use of available scientific knowledge for prevention is becoming increasingly important. Indeed, the authors of the 1972 National Program, recognizing and acknowledging that the major goal of prevention is of necessity the acquisition of new knowledge through basic research, emphasized the demonstration aspects of the mandated Prevention, Control, and Education Programs:

> The overall goal of the prevention and control programs is to benefit the citizens of our country by demonstrating to the practicing medical profession and the public means to promote health, prevent disease, treat disease, and restore health.
>
> Public awareness and access to current information are fundamental to the prevention and control of many specific diseases. . . .
>
> A major program effort is . . . required to disseminate information and to educate the public about . . . disease problems.
>
> The professional community--research scientists, physicians, and related health personnel--also require current and up-to-date information about these disease areas.

The need for effective communication of information to the public and to health care professionals is identified by implication in the stated task that the overall goals of the education and information programs are to:

> make the public aware of the magnitude of these disease problems, the associated risk factors, and available methods of therapy [and]
>
> keep the health professional community abreast of new information and techniques and, thereby, also to

facilitate the flow of modern therapy and information to the public.

At the NIH, the transfer of information to the public and to the professionals who deliver health care is customarily achieved through conferences, workshops, seminars, consensus development meetings, demonstration projects, and publications. (The conferences, workshops, seminars, and consensus development meetings that the DBDR has supported are listed in table 39 following this section.) The authors of the 1972 National Program recognized that the Institute was already engaged in many activities and programs related directly to education and information and that, traditionally, the Institute made available to the public and to health care professionals brochures, pamphlets, and booklets on various heart, blood vessel, lung, and blood diseases and blood resources. The DBDR also disseminates information to professionals and to the public through special programs, such as the Specialized Centers of Research in Thrombosis, the Comprehensive Sickle Cell Centers, the National Research and Demonstration Center in Blood Resources, and the Cooperative Study of the Clinical Course of Sickle Cell Disease.

Transfer of information to the general public as the primary audience, however, represents a different kind of challenge. Opportunities for creative approaches are possible, and the NIH has helped develop a number of successful efforts of this kind. Informing selected, at-risk groups about ways of controlling a disease is generally considered to be within the mandate of the NIH, and the DBDR has developed mechanisms for doing so, particularly in the area of genetic hemoglobin abnormalities. Giving particular attention to teachers, social agencies, insurance companies, and the public, the Division participates in training courses and conferences and responds to specific requests.

To continue and expand the educational aspects of prevention, the DBDR should devise ways to emphasize the integration of its prevention program with its extramural research programs. The Division should consider a series of seminars to enhance the awareness of the staff about the concept, purpose, and means of accomplishing prevention activities. Among the objectives would be to examine ways to use existing knowledge about blood diseases and blood resources to improve health through education. Where appropriate, ways should be explored to determine the existence of reliable prevention-related information. The DBDR should also actively promote new strategies for increasing the public's awareness of health and of the means of arresting or reversing disease.

Eventually, the Division should undertake feasibility studies of ways to transfer information about all relevant prevention-related matters. DBDR efforts in this area could embrace a number of creative approaches.

While some of these approaches may not be possible because of fiscal or other constraints, a few are offered for consideration. Television, for example, is clearly a powerful medium of communication, and it probably delivers more information, good and bad, about health and disease than any other medium. The average viewer probably spends about as much time watching television as he does in engaging in any other leisure activity.

Experience leads to the conclusion that much of the information related to health and disease is distorted and is potentially or actually harmful. As Robert H. Moser of the American College of Physicians observed,

> just as adults learn their medicine from Welby and Gannon, our children are the myrmidons of the lying eye of television. They are manipulated by the ceaseless, senseless, tasteless barrage of deceit from fatuous commercials that lie to them about everything from soap to automobiles. It teaches them that minor pain, fatigue, and unhappiness are not to be endured but can be cured by popping the proper brand of pill. (Sounding Board: "Knowledge is Not Enough," New England Journal of Medicine 296:938-940, 1977),

In "Truth in health or show-biz fluff?" Lois DeBakey calls attention to the confusion between truth and entertainment in the promotion of health, and she comments on the responsibilities of appropriate professionals:

> Our print and audiovisual media are sated with misinformation, confusing half-truths, and fallacious advice bordering on, if not actually constituting, quackery--in how-to books, articles, and radio and television programs on diet, exercise, and bizarre methods of preventing and treating heart disease and cancer, to say nothing of "cures" for acne, hemorrhoids, heartburn, constipation, and other ills, major and minor, that befall mankind. If physicians remain silent while uninformed entertainers (whose own lifestyles are often notoriously unhealthful) become "health educators," physicians must not complain when "health" books containing useless or harmful medical advice appear under the superstars' bylines (written by equally uninformed ghost-writers) or when these new "authorities" dispense unsound medical advice on talk shows and elsewhere.
>
> If the medical community were to use the public media effectively, its designated authorities would become equally recognizable to the public; physicians can be "folk heroes" from whom group habits are acquired. A caption specifying the credentials of the medical

> authority speaking on television would identify the spokesman and give credence to his message. . . . As for sorting out the "distinguished members of the medical community" from the "white-maned, white-coated hucksters that push the patent medicines on the big tube," the current image of physicians is so poor that Madison Avenue has largely abandoned white-coated hucksters for "ordinary people" and your friendly neighborhood drugstore clerk. This year, a make-believe physician, Jack Klugman of "Quincy," was even selected over an authentic physician as a commencement speaker for a medical school.* (American College of Physicians Observer 1:5-6 and 19, 1981)

Moreover, whether they are authentic or portrayed physicians, they may give the impression that a physician, a powerful influence, can do anything needed to restore the viewer's well-being. As a result, the viewer neglects the development of proper health habits. For example, the consumption of alcohol is widespread in television soap operas and prime time viewing, yet alcoholism is noted in only a disproportionately small percentage of characters portrayed on television. Such dramatization leads to the faulty impression that alcohol can be readily managed and is not dangerous.

In addressing the responsibilities of appropriate professionals in providing public-health education, Dr. DeBakey also alerts those who support research and those who conduct it:

> If you abdicate your responsibility for public health education, don't complain if health cultists, charlatans, nostrum-peddlers, and other frauds falsify, pervert, and distort health information for personal gain. You have sanctioned them by your silence. Consider all the hokum "health" books deluging the market today--with unsubstantiated advice about exercise, nutrition, sleep, and stress. You can continue to observe the dissemination of questionable health information and watch your public image being unfairly pilloried--or you can recover your rightful forum and mount a vigorous campaign of health education that serves the public. (Ibid.)

Perhaps the charge is beyond the means and influence of the biomedical community, particularly for commercial television, but there are other, more realistic places where one might begin.

*After this article was published, Mr. Klugman appeared before a congressional committee as a "medical expert."

Cable television, for example, might provide an opportunity for delivery of improved health news. Currently, many cable television stations need material to fill their daily schedules. Many regional charters for cable television require a minimum of public-service and health-information programming. While such time on cable television is likely to be offered without charge, appropriate experts and health groups should be responsible for production and associated costs. With proper program planning, imaginative and attractive health messages can be presented that are more accurate than those implied or suggested by many commercial television programs.

Physicians, who are among the primary audience to which the DBDR directs, or demonstrates, its latest information about innovations for medical practice, have important responsibilities in the dissemination of information about health and disease. Similarly, the community of research scientists must aid in the process of informing the public and health care professionals about new advances and about the importance of the scientific method in achieving them. In fact, as a result of news-media coverage, the public is now being informed about research and developments in health-related fields, and they have a conviction that they should know about the diseases that affect them. Thus, the concept of the physician as an intermediary in disseminating health education must be nurtured. The importance of this concept was exemplified in a recent article by a group of health care professionals at the Duke University Cystic Fibrosis Center (New England Journal of Medicine 305:1278-1280, 1981). In a survey of the 240 patients cared for at that Center, patients and their families wanted to be among the first to know of any new theories or discoveries bearing on their disease. A number of patients wanted to know why they had not heard about a relevant new hypothesis before they saw it on television.

How can scientists and physicians be motivated and stimulated to communicate information relative to prevention to the public? The three obvious means are not equally attainable, although all are possible: publication, cooperation with other health-education organizations, and modification of the scientific and medical curricula.

Communication through publication is not as simple as might be imagined. Innovative methods are needed for obtaining and transferring correct information and for correcting misinformation. A medically and scientifically valid periodical directed toward the public would be effective for those who are already interested in obtaining such information. A broader and perhaps more realistic approach is through the medium of television. Indeed, some commercial stations, such as Channel 7 in Washington,

D.C., as a routine feature of evening news programs, have begun to schedule physicians to discuss recent biomedical developments and to correct misinformation.

A number of organizations involved in health education such as the National Center for Health Education in San Francisco are involved in the delivery of health education to students and employees. As has been demonstrated for many years, health education in primary schools improves the personal health habits of students. Many high school students become keenly interested in health projects. Adequate and appropriate reference sources, such as those available from the Center for Health Promotion and Education of the Centers for Disease Control, meet an important need for students and teachers.

The concept of prevention as an integral part of scientific research and medical practice should begin in graduate and medical school, although implementation of the concept would necessitate modifications of scientific and medical curricula. Academic career opportunities in prevention should also be emphasized, and scientists and physicians should be encouraged to assist in bringing the results of basic research to fruition in terms of the prevention of disease and the delivery of the best possible health care. The entire thrust of prevention might be strengthened by more direct involvement of medical schools. For example, a medical school might establish links between the school and the community that provide the framework for formal programs for dissemination of information about health and disease.

A separate subject is who identifies the information to be transferred or determines whether the information is valid and of potential use in medical practice and in the cultivation of good health habits in the general public. Here, the Division must function as the identifier, the initiator, and the facilitator. It supports the basic research in the field and the clinical trials that validate new methods of medical intervention and disease prevention, and it sponsors consensus development conferences on the appropriateness or efficaciousness of new techniques of intervention and prevention. It is only through knowledge about the content and progress of the research projects and programs of the DBDR that the total, sequential process of information transfer can be accomplished.

Activities, Progress, and Accomplishments

In 1974, two years after Public Law 92-423 was passed, the First Report of the Director, NHLI, presented the following

progress and promise in the area of "Prevention, Control, and Education":

> Prevention, rather than treatment, of heart, blood vessel, lung, and blood diseases offers the greatest promise of reducing death and disability in the United States. More effective application of existing knowledge will materially reduce disability and death from the complications of these diseases. The Institute has provided for expanded future emphasis on bridging the gap between research findings and clinical applications by establishing an Office of Prevention, Control, and Education. An Assistant Director for Health Information has been recruited from within the Institute. This position is mandated by Section 413(d) of Public Law 92-423. The function of this office will be to expand and coordinate the various NHLI programs directed toward the goals set forth in this section of the Plan. Identification of priority areas is already underway.

The report then turned away from further consideration of prevention and control as such and focused on public and professional education:

> . . . [after] consultation with authorities in educational technology and motivational analysis, the Institute has begun planning for a more effective national program in public and professional information and education designed to implement five overall actions in the Plan.
>
> > Develop education programs for each of the categorical program areas of the Institute.
> >
> > Develop better mechanisms, using all media of communication, to disseminate knowledge about heart, blood vessel, lung, and blood diseases and blood resources.
> >
> > Establish improved means for direct communication with the public.
> >
> > Expand programs to aid health professionals in improving the public's understanding and application of new health knowledge and concepts.
> >
> > Establish improved means for communication with the scientific community and the medical profession.

The overall goal of the Prevention, Control, and Education Programs of the Institute is to hasten the control and, in the long run, to prevent or lower the prevalence of cardiovascular, pulmonary, and blood diseases in the population. The emphasis will be on prevention of disease, but in order to achieve optimal success, it will often be necessary to aid the existing health care delivery system in detection of individuals at high risk for a variety of diseases. Improved detection will depend in part on increasing the level of awareness within the population about risk factors, how to enter the health care system, and the importance of patient adherence to management regimens once diagnosis has been established. The educational components of the Institute's control programs will receive the highest priority.

As resources become available, NHLI intends to provide funding for local and regional demonstration programs of prevention, control, and education . . . . Shorter-term demonstration projects are also envisioned as part of the Institute's programs. These demonstration projects will deal with specific techniques . . . not currently accepted or commonly in use for disease control. The intent of a demonstration project should be to provide clear evidence that the new technique is applicable to a community situation, does not overload the existing health care system, is cost-effective, and results in an improved ability of the local health care system to respond to the need which the new technique addresses. Once the demonstration project has confirmed the feasibility, . . . [the] results will be made available to the medical community at large by description in national publications, through National Research and Demonstration Centers, and through voluntary and other health organizations. . . . Other examples of demonstration projects might be new approaches to continuing education of physicians in cardiovascular and pulmonary subjects . . . .

. . . the highest priority program areas are hypertension, arteriosclerosis, inhalation lung diseases, and sickle cell disease, in which modest funds are currently available for educational programs. In addition, as resources become available, new efforts need to be directed to cardiac rehabilitation and emergency cardiac medical services, nutrition and coronary artery disease risk factors, early diagnosis (including prenatal diagnosis) of respiratory distress syndrome in the newborn, emergency treatment of respiratory failure secondary to

> severe trauma, smoking as a risk factor, and improved approaches to blood component therapy . . . .

The Blood Diseases and Resources Advisory Committee in its 1976 report perceived PEC in the context of an educational program for health care professionals and the public. (Subsequent conferences, workshops, and symposia associated with these efforts are listed in table 39.) In reporting on progress in PEC, the Committee noted that these programs in the blood area had been conducted in the fields of blood resources and sickle cell disease.

Blood Resources

In the 1972 National Program, a need was identified to encourage effective and economic utilization of the nation's blood resources. The goal was to improve their management by disseminating information and by educating the primary users of these resources. This concern led to one recommendation for action, which was to

> support continuous physician education regarding optimal use of blood components.

In its 1976 report, the advisory committee stated its expectation that the then newly established National Research and Demonstration Center in Blood Diseases and Resources would include the education and demonstration aspects of managing the national blood resource. Mention was also made of the Division's cooperation with the American Blood Commission in determining priorities in prevention, control, and education programs and of the expectation that a national blood data center would have a major impact on planning and education in blood resources. The advisory committee's recommendations in this area were the following:

- Important to the success of the National Blood Program is the development of an effective demonstration process. Further education will be required of scientists and others involved in health care as to the nature of the demonstration process: taking something which is shown to be scientifically sound by laboratory research or field trials and showing that it is an effective component in the health care system. Judgment is required as to which innovations require demonstration as compared to those which would readily be implemented by the existing health care system without such assistance.

- A support system to deal with demonstration projects needs to be developed which utilizes the strength of the formal grant proposal, study section review, and competitive renewal. Much more emphasis should be placed on the

suitability of the project than with the research grant, but the same necessity exists for clear objectives, measurements which are adequate to determine the effects produced, and suitable dissemination of information.

- There is need for a highly coordinated public education program on the need and value of volunteer blood and other organ donations. In addition, programs are required to educate physicians in the proper use of blood and blood components. There is a need to further acquaint the physician with the potential infectious hazards, particularly viral hepatitis, of transfusion of blood and blood derivatives according to source or manufacturing process.

- The Institute should continue to play a leading role in collaboration with the ABC to determine future priorities in the development of blood resources and the prevention, control, and education programs required for fostering a high quality national blood program.

- Research and training programs and the special interests of blood resources should be expanded and integrated with those of the general hematology program.

The primary strengths of the Division's National Research and Demonstration Center are in basic and clinical research. Activities of the center are community-based, and that environment has offered opportunities for demonstration and education not otherwise available. The center has demonstrated, for example, the feasibility of using cryoprecipitate as a major resource for the treatment of some hemophiliacs. In addition, it has monitored and analyzed, thoroughly and on a large scale, the practices of hospitals and physicians in ordering blood for transfusions.

The problems for which the American Blood Commission was established to address have been largely resolved. The use of blood from paid donors for transfusion declined dramatically when studies demonstrated clearly that such blood was associated with very high rates of hepatitis. Regional, long-term, as well as seasonal, shortages of blood were reduced by a combined approach to the problem: more effective donor recruitment methods were used by blood centers; interregional shipments of blood from surplus to shortage areas were expanded; inventory management and distribution practices by hospitals and blood centers were improved; and component therapy, which expanded the usability of supplies of available blood, was more widely applied. In addition, a newly formulated preservative (CPD-adenine) keeps whole blood and red cells usable for 35 days after collection, in comparison with a previous 21-day limit. There are some problems yet to be resolved concerning the recruitment of adequate numbers of donors from various ethnic groups in large metropolitan areas

and in ensuring that blood therapy provided to patients is optimal, but the solution to these problems is dependent upon results of future research.

Details of research accomplishments of the blood resources program can be found elsewhere in this report. Some of them are so closely related to current concepts of prevention that they deserve highlighting here. Research on methods for separating and preserving blood components and on means for evaluating their clinical efficacy permits exploration of the use of blood components for the treatment or control of certain diseases. The results of a prophylactic trial of granulocyte transfusions in leukopenic patients, for example, indicated that episodes of bacterial sepsis were decreased and the incidence of other infections was not affected, but pulmonary infiltrates were increased. It was therefore concluded that granulocytes given prophylactically could not be recommended as standard therapy during leukopenic states, and as a result of this and similar clinical trials, the use of granulocytes in at least one form of therapy has decreased.

Apheresis, a general term that describes the removal of a selected component of vascular fluid, has become an increasingly used technique. During the past decade, the administration of platelet concentrates, for which the demand continues to increase, has become an essential adjunct to cancer therapy, and it has been facilitated by plateletapheresis techniques, which permit the rapid collection of large numbers of platelets. Cytapheresis, a process that removes certain white blood cells from the blood of a patient, has benefited some patients with leukemia and other diseases who fail to respond to platelet transfusion as a result of a reaction characterized by platelet destruction. The HLA system, important in transplantation biology and first brought to clinical prominence because of its selection potential for donor-recipient pairs for kidney grafting, has been found to be important in preventing such reactions. Platelets from HLA-matched donors combined with techniques of cytapheresis offer the best therapeutic hope for sensitized recipients. In cytapheresis, an extracorporeal circuit is established, a donor's blood is centrifuged, and selected cells are harvested; the remainder of the blood is returned to the donor. Application of this procedure permits the collection of a therapeutically adequate amount of platelets or leukocytes from one HLA-matched donor. Plasmapheresis is a technique used almost exclusively for the collection of source plasma for fractionation into various plasma derivatives. Therapeutic aspheresis, which is the selective removal of certain cellular elements or plasma constituents from the blood of a patient, has been encouragingly successful in the treatment of some diseases. The procedure is still experimental, however, and is the subject of continuing investigations.

In the area of immune globulins, the use of Rh_o(D) immune globulin, for which the primary indication is prevention of postpartum sensitization, has been extended to include administration to Rh-negative women after miscarriage or amniocentesis. In addition, two new immune globulin products have become available during the last decade. One type is made up of specific immune globulins for intramuscular use, such as rabies immune globulin, which was introduced in 1974, hepatitis B immune globulin, in 1977, and varicella-zoster immune globulin, in 1980. The second type, introduced in 1981, called "Immune Globulin Intravenous," is the only human immunoglobulin product for intravenous use currently on the American market. The advantages of intravenously administrable immune globulin, as compared to the conventional intramuscular product, are greater comfort for the patient, rapid attainment of high titers of antibodies in the circulation, and capability of being administered in larger doses.

The hazard of blood transfusion--the transmission of the elusive non-A,non-B hepatitis virus(es)--is complicated by the inability to detect the virus(es) by use of current tests for hepatitis. The prevention of transfusion-transmitted virus diseases, however, is nearer reality as a result of research on methods for detecting the virus(es), on new techniques for inactivating potentially infectious viruses, and on the development of blood substitutes.

Researchers have succeeded in transmitting some forms of hepatitis from the blood of infected humans to chimpanzees. The transmission of hepatitis to experimental animals has provided investigators with a much-needed animal model for studying the disease.

In the case of hepatitis B, an interim means of prevention is the use of hepatitis B immune globulin, which, if administered within 7 days after acute exposure and again about 1 month later, can reduce the incidence of hepatitis B. At present, hepatitis B immune globulin is indicated for prophylaxis after either exposure to an infected member of the family, direct mucous membrane contact, or oral ingestion involving infectious materials such as blood, plasma, or serum.

Among the most exciting advances in prevention is the development of the hepatitis B vaccine. As a result of a successful efficacy trial, the Food and Drug Administration has recently licensed this new inactivated hepatitis B vaccine, the first to reach American physicians in 10 years. Among the first designated for vaccination with the product are physicians, dentists, nurses, and other groups for whom exposure to hepatitis B is frequent.

In a related project supported by the Division, the investigators prospectively monitored recipients of blood, as well as

controls not receiving transfusions, in four cities during the period from July 1974 through November 1979. Thus, a comprehensive indication of the risks of viral hepatitis associated with hospitalization and with transfusions is now available.

Blood substitutes have particularly exciting potential for intervention. Once developed to the point that they can be used easily in humans, these preparations, which would supplement and conserve natural blood supplies, should be invaluable in times of acute shortages or widespread disaster and could be lifesaving in instances where natural blood is undesirable. In addition, with these artificial materials, it should be easier to preserve organs for research or transplantation than with normal blood. The intervention procedures that can be made possible by effective blood substitutes are limited only by the imagination. Preparations containing perfluorochemicals, for example, have been used successfully to maintain for days needed oxygen tension in extracorporeal circulation procedures. Thus, mixtures containing perfluorochemicals can be used effectively to maintain isolated organs such as heart, liver, kidney, brain, and skeletal muscle during reparative procedures or during specific forms of therapy.

In cases of myocardial infarction, the increased P_{O_2} values that can be obtained with perfluorochemical preparations has led to their use in the experimental treatment of tissue anoxia. In experimentally induced heart anoxia in dogs, the areas of post-anoxia damage were significantly reduced when a perfluorochemical emulsion rather than blood was administered. In other studies, perfused hearts subjected to anoxia responded better to cardioplegic solutions containing emulsified perfluorochemicals. In brain-anoxia experiments, better results have been obtained when emulsified perfluorochemicals were used during recovery.

Sickle Cell Disease

In the 1972 National Program, the authors were of the opinion that the major "control" programs, which they envisioned as being accomplished through education programs, were in sickle cell disease and Cooley's anemia:

> The goals of the control program are to educate the public about sickle cell disease and Cooley's anemia and to demonstrate techniques for appropriate screening and counseling.

The opportunities and goals included the need to determine the prevalence and distribution of sickle cell disease and Cooley's anemia in the United States, particularly the prevalence of the trait; to provide current and accurate information on these diseases; to initiate, continue, and expand appropriate community

education, screening, and counseling programs; to inform the medical and other health care professionals about these diseases; to identify and provide advice on the management of the psychological, sociological, and economic aspects of these diseases; and to encourage patients with them to attain their maximum potential in life. The recommended actions to meet these needs were to:

> develop, in collaboration with other federal agenices, programs to provide accurate information on sickle cell disease and Cooley's anemia; education for the general public, population groups at risk, health professionals, allied health professionals, educators, employers, and insurers of individuals who have these disorders; methods to best counsel affected individuals; and proper rehabilitation approaches when applicable; [and] evaluate clinical approaches for the treatment of these disorders and ensure that the ones that work are incorporated into the health delivery system.

In the 1976 report, the Blood Diseases and Resources Advisory Committee attributed the accomplishments in the area of sickle cell disease to the activities of the 24 sickle cell clinics administered by the Health Services Administration, with substantial funds also provided by the NHLBI and the 15 Comprehensive Sickle Cell Centers administered by the Division. These accomplishments included

> . . . a service for the dissemination of information about this disease and . . . education for physicians and related health personnel, [and] . . . education and counseling techniques developed according to the needs of the individual clinics.

The advisory committee's recommendations for future prevention, control, and education activities in sickle cell disease were the following:

- Now that screening, counseling, and education programs are in operation there is need to evaluate their effects on the population served. It is recommended that more specific objectives, protocols for data collection, and plans for evaluation be developed through which the specific achievements of this program can be identified.

- Activities in screening, counseling, and education at the various sickle cell centers should be evaluated so that information can be gathered as to the impact of various programs.

- An assessment of the effect of genetic counseling on the population counseled is of high priority since results are

not necessarily all positive and since there is considerable question at this time as to the overall effect of this process.

- The broad impact of this program on health care in the community where such operations are carried out should be studied. One aspect to be evaluated is the effect of this program on health care personnel and health services in improving the care of patients with sickle cell disease (and Cooley's anemia). Even more important is the effect of the program on the well-being of affected individuals, both those with the disease and with the trait, and on the incidence of the disease. The intervention provided by this program may serve as a prototype for other chronic hereditary and acquired diseases for which there is no specific therapy.

- When screening clinic activities have been standardized in respect to laboratory techniques, genetic counseling methods, and education programs, the orderly transfer of these programs into established institutions responsible for control, health maintenance, health care, and undergraduate and continuing medical education should be undertaken.

The evaluation called for in the first recommendation has been completed. Results of the study revealed that, in the educational aspect, public awareness of sickle cell disease improved in geographic areas where there were Federally funded programs, especially when these programs were in the form of Health Services Administration's Genetic and Sickle Cell Testing and Counseling Projects. The evaluation and assessment called for in the second and third recommendations are currently being completed. The activity generated by the fourth recommendation is an ongoing process. One of its notable results thus far focuses on the effect that the sickle cell disease program has had on improvement in the care of patients with sickle cell disease. Powars, Overturf, Weiss, Lee, and Chan (Journal of the American Medical Association 245:1839-1842, 1981) reported that a decrease in major morbidity among children with sickle cell anemia can be attributed to the establishment of a clinical program that provides close medical supervision of the child with sickle cell anemia and allows the rapid institution of parenteral antibiotic therapy when necessary.

There has been considerable activity in other areas related to the education of public and health care professionals with regard to sickle cell disease. This activity had its origin in the President's Health Message of February 1971, which highlighted sickle cell anemia as an important health problem. This speech was the initial introduction of most Americans to the existence of

this health concern and, in fact, was interpreted by many as the announcement of the discovery of a new disease. As a result of the dearth of accurate educational materials related to sickle cell trait and anemia at that time, the information available to the general public was inaccurate and confusing, and it generated misconceptions, stigmas, and anxieties. A review in 1973 by the National Association for Sickle Cell Disease of the published information on sickle cell disorders for lay distribution highlighted the magnitude of the problem. Surveys in local communities documented that many members of the general community lacked understanding of the difference between the carrier state and the disease; further, it was viewed by some as being contagious and by many as occurring only in blacks.

The net effect of inaccurate information was the arousal of anxiety, fear, and apprehension; the generation of guilt, resentment, and frustration; and inhibition of the development of personal self-esteem and racial pride. In fact, attitudes of potential employers about the extent of incapacity and disability accompanying sickle cell disease often resulted in unjustifiable hiring practices. Persons with the trait also experienced difficulty in securing certain positions. The airlines and the military, for example, raised questions about their fitness for certain types of duty. Appropriate education is essential for preventing discrimination and needless unemployment among persons with the disease. The educational programs conducted by the sickle cell disease program, the comprehensive sickle cell centers, and the screening and education clinics were directed to the prevention of these economic and psychosocial ramifications.

The existence of an interested, albeit misinformed, general community emphasized the need for practicing physicians to be well informed about sickle cell trait and disease, especially since evidence was accumulating that demonstrated how poorly informed some medical professionals were about the disease. An evaluation in 1974 of physicians' attitudes and knowledge revealed that 15 percent of them believed sickle cell trait to be commonly responsible for illness. An evaluation of emergency room care for sickle cell patients at a large city hospital revealed that the quality of the care was inadequate and in some cases even detrimental. The fact that clinical manifestations were erroneously diagnosed, not recognized, or improperly managed emphasized the need to provide adequate education to health care professionals if serious errors in diagnosis and management were to be prevented.

Teachers, school counselors, psychologists, and special education consultants for home instruction and vocational training who are poorly informed about sickle cell disease are unable to develop effective programs to ensure a successful educational experience for children who have the disease. Misconceptions regarding the characteristics of the illness, including mental

capacity and physical activity, can harm a child's awareness of self and prevent development of a sense of competence, mastery, and self-esteem. Education of school officials is therefore an important form of prevention, for it reduces frustrations and feelings of inadequacy that impede a child's progress in school and can eventually cause rejection of school.

An important part of the educational effort is the counseling of patients who have sickle cell disease and of those who have the potential for producing offspring with the disease. Essential for counseling, of course, is detection, for which screening methods have been developed that permit accurate identification of carriers of abnormal hemoglobins. Couples at risk can now be identified, and they can freely discuss family planning choices at a time when such decisions are most important. Counseling programs conducted in the comprehensive sickle cell centers, as well as in the screening and education clinics administered by the Health Services Administration, facilitate effective communication between the patients and their families and enable people to make informed decisions with respect to marriage and family planning. These counseling sessions provide accurate and definitive information about the medical and genetic aspects of the disease, correct misconceptions, and give emotional support and assistance. This aspect of genetic counseling prevents a lack of choices in important personal decisions.

Another important area of prevention is prenatal diagnosis of the disease. Early approaches to prenatal diagnosis required the use of fetal blood for direct analysis of the small amounts of beta chains, a procedure that posed considerable risk to the fetus. A small flexible fetoscope has now been developed that enhances the accuracy and safety of the procedure. Major advances have also been made through the use of recombinant DNA technology. Prenatal diagnosis by means of gene mapping of DNA obtained from amniotic-fluid fibroblasts is now possible with amniocentesis, a procedure widely available throughout the country with minimum or no risk to mother or fetus. Recent improved techniques for identifying the point mutation at the beta-6 position of the globin gene make this technique applicable to all pregnancies at risk for offspring with homozygous sickle cell disease.

Newborns can now be screened for sickle cell disease and other relevant hemoglobinopathies with the use of cord-blood samples. This technique offers an opportunity for early preventive therapy. Through immediate entry into the medical care system, more effective management, alertness of the health care team to potential problems, and education of parents, it is now possible to prevent many of the often fatal complications of infections of children with sickle cell disease. The recognition of sickle cell disease in the newborn also provides the opportunity to provide early counseling to the parents about the status of the child's

disease and permits the discussion of alternatives regarding future pregnancies.

A common cause of morbidity and mortality in children with sickle cell disease is their particular predisposition to pneumococcal bacterial infections. The fulminant characteristic of pneumococcal infection compromises the effectiveness of antimicrobial therapy, and the most common cause of death in such children under 2 years of age is from overwhelming pneumococcal septicemia. Although the etiology of increased susceptibility to infection remains poorly understood, prevention is the best approach to managing this complication.

Investigations focused on the prevention of this complication have included two approaches: one was the use of prophylactic penicillin therapy on a daily basis to prevent or ameliorate the severity of the infection, and the other was the administration of pneumococcal vaccine to young infants to enhance development of specific antibody against the pneumococcal organism. Although both approaches represent advances against the most common cause of death in children with sickle cell disease, the polyvalent pneumococcal vaccine, which is ineffective under age 2, has been only partially successful (60 to 80 percent) in preventing this major complication.

To prevent further complications associated with sickle cell disease, physicians often have to administer multiple blood transfusions to patients. The transfusions provide temporary improvement of the chronic anemia, reduce the percentage of sickle hemoglobin in the circulation, and, for a few patients, reverse abnormal changes in cerebral arteriograms and reduce the recurrence rate of strokes. Recently, hypertransfusion of pregnant women who have sickle cell disease has been found by some physicians to prevent complications associated with pregnancy and to improve the well-being of mother and infant. This concept is analogous to the long-term use of penicillin to prevent recurrence of acute rheumatic fever in children; it is not considered to be therapeutic.

The value of prophylatic blood transfusions to prevent specific complications of sickle cell disease remains controversial, and it is approached cautiously because of the possibility of iron overload, transmission of hepatitis or other infections, development of antibodies against minor red cell groups, and febrile reactions secondary to contamination with leukocytes. A consensus development conference on the topic of this approach to management in pregnancy, as listed in table 39, recommended further research to assess the benefits and risks of this mode of prevention.

It has been well established that long-term chronic disorders can lead to a social disability that may be far more serious than

the physical problem. Thus, it has become increasingly important for the DBDR to encourage educational programs that include psychosocial assistance and emotional support to prevent feelings of insecurity, inadequacy, and hopelessness while promoting a realistic outlook for the future and improving the quality of life. Tutorial and vocational rehabilitation programs are being assessed for their effectiveness in increasing scholastic achievement, decreasing school absenteeism, increasing self-esteem, and decreasing the psychological pressures of adaptation.

The DBDR has provided other services, including evaluation and compilation of educational materials; participation in workshops, conferences, and symposia; presentation of exhibits at conventions and conferences, and a permanent exhibit in the health sciences sections of the Chicago Museum of Science and Industry; responses to requests for information; development of a directory of sickle cell programs; provision of technical assistance to community programs; and coordination of educational and informational programs across Federal agencies. Many information publications, including versions in several languages, have been developed and disseminated to professional and general audiences. A self-instruction audiovisual kit for physicians has been produced that contains information about the symptoms, diagnosis, and physiological and psychological problems associated with sickle cell disease and sickle cell trait. The kit has been disseminated to 6,000 black physicians.

A rather unusual training device, referred to as the Health or Science Teacher Training Seminar program, was designed to introduce concepts about sickle cell disease to high school students, through training of their teachers, and to encourage regional, state, and local organizations to promote similar educational programs. Seminars have been held in major cities throughout the country. Preliminary evaluation of these seminars indicates that the sessions were effective in increasing knowledge about sickle cell disease among the teacher trainees. These teachers should now be able to present to their students valuable information about the characteristics of, and risks associated with, sickle cell trait and the disease.

In addition to these educational efforts, the DBDR has supported numerous scientific research programs that contribute significantly to prevention efforts. Although most of these investigations are discussed in detail elsewhere in this report, a number of them warrant special mention in this context. The results of a multi-institutional, double-blind clinical trial, for instance, demonstrated that intravenous urea solution was no more effective in the treatment of painful crises than was alkalai or glucose. Orally administered sodium cyanate, an effective anti-sickling agent that was often a component of the urea solution, has side effects, particularly neurologic toxicity, which preclude

its clinical use by that route of administration. To circumvent this problem, techniques for continuous extracorporeal carbamylation that have been developed and studied for their ability to maintain effective carbamylation in laboratory animals have now been applied to selected patients with severe sickle cell syndromes, and the feasibility of the approach has been demonstrated. Thus, one may now consider the possibility of treating patients in sickle cell crises with extracorporeally administered cyanate, one of the most promising therapeutic agents discovered so far for sickle cell disease.

Eye complications in sickle cell disease have been documented through clinical observations and research. New photographic contrast techniques have been developed, and a standardized classification of the evolution of sickle cell retinopathy is now available to the practicing physician. Early diagnosis and proper referral can significantly reduce the potential for blindness and other eye complications in sickle cell disease.

An evaluation of emergency room procedures for patients in sickle cell crises, who may experience complications that are often unrecognized and misdiagnosed, led to the development of a protocol that identifies signs and symptoms and suggests various procedures of treatment. This protocol has been made available to emergency rooms in medical centers and hospitals throughout the country.

Blood Diseases

In addition to the recommendation related to Cooley's anemia, as included with sickle cell disease, the section on Prevention, Control, and Education of the 1972 National Program included one additional subject in blood diseases: hemophilia. The consultants believed that the existing genetic counseling was not working and that the hemophilia population was likely to increase. The goal they considered for the prevention effort in hemophilia was an educational program to help hemophilia carriers and patients make informed decisions about their own lives. They therefore urged establishment of a strong, positive program of genetic research and counseling. The recommended action was to

> develop information, education, and genetic counseling services aimed at better treatment and prevention.

Hemophilia was also the subject of a discussion by the Blood Diseases and Resources Advisory Committee. In its 1976 report, the committee identified opportunities for educational and training functions that are, or could be, available in comprehensive care centers, but it did not make specific recommendations for prevention, control, or education. This present report goes far

beyond the 1976 advisory committee, which emphasized two areas; it considers the progress in the PEC program in all program areas of the DBDR.

Studies supported by the Division exemplify research that can be related to the goals of the PEC program. The systemic infusion of activators of fibrinolysis--the thrombolytic agents streptokinase and urokinase, which have been shown to be effective in dissolving venous clots and pulmonary emboli--may lead to the immediate or eventual prevention of thromboembolic diseases. These drugs are now licensed for general use. They are also now being explored for their value when infused during cardiac catherization for the treatment of acute myocardial infarction. In addition, new techniques have been developed to identify deep venous thrombosis and pulmonary embolism, conditions that are extremely difficult to diagnose. These advances include Doppler ultrasound, impedence plethysmography, ^{125}I-fibrinogen scans, lung scans with isotopically labeled gases, and radioimmunoassays for fibrinopeptide A, platelet factor 4, and beta-thromboglobulin.

Low-dose heparin has been shown to be effective in the prevention of postoperative deep venous thrombosis in certain groups of high-risk patients. Low-dose heparin is also used prophylactically in patients with myocardial infarction. In addition, some groups of patients may be protected from the risk of deep venous thrombosis by use of external pneumatic compression and early ambulation.

In other studies, investigators have shown that aspirin will reduce the incidence of transient ischemic attacks that are related to platelet-fibrin thromboembolism. Antiplatelet drugs in combination with anticoagulants have also been useful in reducing thromboembolism associated with prosthetic heart valves. Although results of studies in certain conditions have been controversial, there is general agreement that antiplatelet drugs, such as aspirin, dipyridamole, and sulfinpyrazone, are useful in the prevention of postoperative venous thrombosis, particularly in patients undergoing hip surgery.

Several in vitro tests of platelet function have been devised and introduced in attempts to diagnose and treat patients with bleeding and thrombotic disorders. These tests can facilitate the description of abnormal platelet function in a variety of acquired disorders and thus have enabled investigators to identify a variety of drugs that interfere with platelet aggregation, adhesion, or secretion.

Thrombotic thrombocytopenic purpura is a rare but important disorder, since it appears to represent a prototype of abnormal hemostasis related to impaired interaction between platelets and

endothelial cells. The treatment and prognosis of this once-lethal disease have improved over the past decade. Treatment now involves plasmapheresis, plasma transfusions, and antiplatelet drugs.

The understanding of the biochemistry and physiology of the factor VIII complex has increased significantly over the past 10 years and has aided in the prevention and treatment of hemophilia and von Willebrand's disease. In addition, the availability of freeze-dried concentrates of antihemophilic factor (factor VIII) has made home therapy a reality for many hemophiliacs. Improvement in the quality of these patients' daily lives has been demonstrated by the decrease in time lost from school or work, by less unemployment, and by increased productivity. In addition, detection of hemophilia carriers has been improved: with a combination of tests for coagulant activity and immunologic reactivity, along with studies of the medical history of families, investigators have increased the accuracy of detection of carriers from 20 percent to about 95 percent. The use of these methods has also improved the detection of the disease in utero. Analysis of blood samples, obtained by fetoscopy, from fetuses at risk for hemophilia provides information that facilitates family planning. These advances have been disseminated in cooperation with the National Hemophilia Foundation through the development of educational movies and slide presentations.

Some hemophilia patients develop inhibitors of factor VIII, which can be a serious complication in the treatment of their disease. Its appearance and characterization in hemophiliacs has been documented in a cooperative clinical study that provides the most complete description available of the complication. The investigators found inhibitors in about 15 percent of the patients and concurrently established new internationally accepted methods for measuring inhibitors. In addition, a prospective, randomized, double-blind clinical trial of the use of prothrombin complex concentrates showed that these concentrates are effective in controlling about 50 percent of episodes of joint bleeding in hemophiliacs with inhibitors. New immunoelectrophoretic methods have been employed to diagnose von Willebrand's disease and to classify its various forms. This information has allowed more effective treatment of bleeding in patients with this disease.

In summary, progress in the prevention of several blood diseases has been encouraging. Additional important advances are discussed elsewhere in this report. In regard to prevention, the most notable advances include the use of aspirin in the prophylaxis of thrombosis; the interaction of platelets with injured blood vessels that can lead to atherosclerotic vascular disease; the measurement of both thrombin and plasmin action in plasma as an aid to identifying patients at greatest risk for developing clinical thrombosis; the discovery of a new pathway of arachidonic

acid release in platelets as an aid to finding antiinflammatory agents; the development of gene-insertion techniques and their possible applicability to the treatment, control, or cure of sickle cell disease and the thalassemias; and the exploration of improved methods of removing excess iron from Cooley's anemia patients who must receive frequent transfusions.

Accomplishments in education for the public and for health care professionals include the preparation and dissemination of two educational booklets, "Cooley's Anemia: Prevention Through Understanding," and its companion, authored by representatives of the Cooley's Anemia Foundation, "Cooley's Anemia: Prevention Through Testing." Within the past year, over a half million copies of "Understanding" have been distributed to schools in areas identified as having populations in which there is a significant risk of Cooley's anemia. Over 2 million copies of the booklet encouraging young people to be tested for the trait have been distributed to schools, fraternal organizations, public health facilities, hospitals, physicians' offices, public and school libraries, and community service organizations and programs. It is much too soon to evaluate, if evaluation is ever possible, the impact of these materials on the "prevention," or reduced incidence, of Cooley's anemia. It is questionable whether distribution of materials unaccompanied by some kind of special promotion or educational activity is an effective technique for achieving the goals of a prevention program.

The number of victims of Cooley's anemia has been a subject of considerable speculation and controversy. The Division's survey of the incidence of Cooley's anemia in the United States has been of significant value in resolving this issue. The study was much more than a survey. The experts who conducted it accomplished five important tasks: they reviewed the status of research on Cooley's anemia; developed practical standards for clinical services and issued comprehensive guidelines for all aspects of treatment as based on current research; determined the actual dimensions of the prevalence of the disease and, by estimation, the frequency of the gene; surveyed the facilities where Cooley's anemia is treated, including treatment practices in the major medical centers where most patients are seen; and studied the impact of the disease on patients and their families. The results of this extensive effort were published as Assessment of Cooley's Anemia Research and Treatment (NIH Publ. No. 80-1653). The report has been in considerable demand, particularly by physicians; it had to be reprinted only 1 year after it was published.

Summary and Conclusions

The prevention-related activities discussed above involve facets of the Division's programs that encompass basic and clinical research, demonstration, dissemination of new information, and education. Moreover, most of the recommendations for specific program areas, as found throughout the report, are also goals for prevention.

Since the advisory committee's 1976 report on the Division's PEC activities, which were identified as only those areas in which public education would result in motivation to seek genetic counseling or to donate blood, the concept of prevention has appropriately evolved to the recognition that most of the basic research supported by the NIH is inherently relative to the goals of disease prevention and control. The Division enthusiastically endorses this concept.

The Division considers its entire program to be related to prevention, intervention, or control. The public statement of its responsibilities affirms this commitment:

> This Division plans and directs the NHLBI's research grant, contract, and training programs to improve the diagnosis, prevention, and treatment of blood diseases and related disorders and to assure the efficient and safe use of an adequate supply of high-quality blood and blood products. (1980 NIH Almanac, NIH Publ. No. 80-5)

Thus, according to the current definitions of prevention and control,* a vigorous and substantive PEC program in the Division's ongoing grants and contracts programs can be identified. In fact, progress and accomplishments in all the Division's program areas are reliable indices of its progress in prevention.

*According to Research in Prevention: Fiscal Year 1980, a portion of the increase in funds for NIH prevention-related research from $352,000,000 in fiscal year 1978 to $506,000,000 in fiscal year 1980 (69 percent increase) "reflects greater agreement as to what constitutes primary prevention research."

Table 39. National and International Conferences, Workshops, and Symposia Supported Fully or in Part by the Division of Blood Diseases and Resources

1974: International Symposium on Blood Substitutes
First National Sickle Cell Disease Symposium
Workshop on the Thrombogenicity of Factor IX Concentrates
Laboratory Workshops on Carrier Testing

1975: Workshop on Animal Models of Thrombosis and Hemorrhagic Diseases
Conference on Molecular and Cellular Aspects of Sickle Cell Disease
Workshop on Prophylactic Therapy of Deep-Vein Thrombosis and Pulmonary Embolism
Workshop on Animal Models of Thrombosis and Hemorrhagic Diseases
Conference on Frozen Red Cell Outdating
Workshop on Albumin

1976: Workshop on Platelet Function Testing
First National Educational Symposium on Sickle Cell Diseases
Conference on Platelet Function Testing
Workshop on Unsolved Therapeutic Problems in Hemophilia

1977: Workshop on the Biochemistry and Physiology of Factor VIII
Workshop on the Thrombotic Process in Atherogenesis
Conference on Biochemical and Clinical Aspects of Hemoglobin
International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation

1978: Conference on Prenatal Diagnosis of Hemoglobinopathies
Conference on Cellular and Molecular Regulation of Hemoglobin Switching
Conference on Development of Therapeutic Agents for Sickle Cell Disease
Conference on the Chemistry and Physiology of the Human Plasma Proteins

Table 39. National and International Conferences, Workshops, and Symposia Supported Fully or in Part by the Division of Blood Diseases and Resources (continued)

1979: Workshop on Therapeutic Plasmapheresis and Cytapheresis
Conference on Immunoglobulins: Characteristics and Uses of Intravenous Preparations
Consensus Development Conference on Hypertransfusion Therapy in Pregnancy

1980: Conference on Prostaglandins in Cardiovascular and Thrombotic Disorders
Conference on Plasma and Cellular Modulatory Proteins
Workshop on Laboratory Evaluation of Platelets for Transfusion
Conference on the Influence of Female Hormones on Hemostasis and Vascular Disease
Conference on Psychosocial Aspects of Sickle Cell Disease
Conference on Regulation of Hemoglobin Switching

1981: International Symposium on Abnormal Hemoglobins: Genetics, Populations, and Diseases
Conference on Prostaglandins in Cardiovascular and Thrombotic Disorders
Conference on Rheological Contributions to Thrombosis and Hemostasis
Workshop on Alterations of Blood Rheology and Micro vasculature in the Pathogenesis of Sickle Cell Disease

1982: Workshop on Sickle Cell Disease
Conference on Factor VIII-von Willebrand Factor
International Symposium on Blood Substitutes
Conference on Coagulation, Cancer, and Inflammation

7. Research Training and Development

Contents

7. Research Training and Development

This section of the report addresses the topic of research training in the fields of hematology and blood banking.* While the mission of the Division of Blood Diseases and Resources at the National Heart, Lung, and Blood Institute includes responsibility for only a segment of the broad discipline of hematology, the discussion is not limited to program areas specifically under its jurisdiction. Training needs in all aspects of blood-related research other than oncology are reviewed, as well as the full range of activities related to the operation of the modern blood bank.

Training and education are parallel and related activities, and they are the concern of scientists throughout their careers. This report considers broadly based research training as well as that which is narrowly focused. Predoctoral and postdoctoral training and continuing education related to the clinical practice of medicine are not discussed. The report focuses on training for careers in basic and clinical research for the MD as well as for the PhD in classical disciplines related to hematology and blood banking. Research concerned with less traditional areas related to blood bank management is also considered.

The DBDR has a legislative mandate to support research in specific program areas belonging to the specialty of hematology. While the level of the Division's support of hematologic research at the National Institutes of Health exceeds that of other Institutes, including the National Cancer Institute (NCI), which has primary responsibility for oncology, these other Institutes provide important sources of support in the blood disease areas. While in most areas, there is no overlap, significant sharing does occur in other areas. The major burden for the support of research training in hematology at the NIH, however, falls to the

*This discussion represents the thinking of DBDR staff who work in conjunction with educators active in hematologic training programs. In addition, key scientists involved in a variety of studies of training during the last decade contributed their expertise to this effort. No formal committee on training existed at the time of writing. These ad hoc consultants carefully reviewed the material prepared by staff, and their ideas are fully represented in the final document. (See appendix B.)

DBDR, which currently provides funding for approximately three-quarters of all nononcologic trainees in this field (two-thirds of all blood-related trainees if oncology is included). Blood banking research and related research training at the NIH are the sole responsibility of the DBDR. The present discussion is applicable not only to blood banks but also to much of hematology, even though a specific research objective might be funded by an Institute other than the NHLBI.

The instruments of training support at the NIH have evolved gradually. A detailed summary of these developments is provided in appendix A. The first mechanisms were the research fellowship and the clinical traineeship. Circumstances at that time made it advisable to establish the latter to train additional cardiovascular physicians so that they could apply research and technological advances. The clinical traineeship was terminated in 1958 since it appeared to have achieved its goal (see figure 8 in appendix A).

The predecessor to the graduate training grant appeared in 1948, and this kind of grant has continued to evolve into its present complex multi-purpose form. In 1960, special programs, such as the special fellowship and part-time (summer) fellowship, were initiated and encompassed within the general research support grant. In 1961, the research career award program was initiated to provide for increased numbers of stable academic research careers.

Training in the 1970's was marked with uncertainties about continuing legislative authority and appropriations. The temporary abolition of all training programs in 1973 and the rapid reinstatement of comparable programs by Congress in the National Research Service Awards (NRSA) Act of 1975 confused an already uncertain biomedical research community. An important development during this decade was a shift in national priorities from training for academic research careers to training for larger participation in health care delivery.

These factors, individually and combined, undoubtedly discouraged young scientists, particularly physicians, from considering academic research careers. To entice this group into such careers, the clinical investigator award was developed. It provides a bridge between the postdoctoral clinical period and a postdoctoral research training experience in a categorical disease area. The short-term (summer) fellowship was reintroduced in 1980 to provide medical students with a structured research training experience of up to 3 months.

Increased efforts will be necessary during the 1980's to recruit and maintain an adequate number of investigators, since

the scientist trained in this decade will make the research advances of the 1990's.

Hematologic Training 1972 to 1982

On July 14, 1972, the Department of Health, Education, and Welfare (DHEW) approved a reorganization of the then National Heart and Lung Institute that elevated the Institute to bureau status within the National Institutes of Health and created the Division of Blood Diseases and Resources. This action recognized and renamed the precursor of the DBDR, which was the NHLI thrombosis and hemorrhagic disease program. Through it, the NHLI had supported blood research since August 1969. As a result of P.L. 94-278, passed in 1976, the name of the Institute was changed to the National Heart, Lung, and Blood Institute. The change emphasized the Institute's responsibility to support hematologic investigations related to blood diseases, blood derivatives, and the management of blood resources.

Developments in the National Institutes of Health and the National Heart, Lung, and Blood Institute

The National Heart and Lung Advisory Council recognized the importance of training by stating in the National Heart, Blood Vessel, Lung, and Blood Program, which was developed in 1972:

> Supply of skilled manpower is an essential element in a national effort designed to advance the attack upon heart, blood vessel, lung, and blood diseases. An adequate long-range plan in an expanding program directed against these diseases will require an expanding manpower pool to conduct research and to replace skilled manpower lost through attrition.

The Council stressed the importance of strengthening and providing continuity to the various support mechanisms available for recruiting and training talented young scientists. Because of this need, and in order to encourage investigators to explore new areas of research, the Council recommended the support of professional research groups and the creation of a variety of training and development awards such as research professorships, research career development awards in special areas, and special research fellowships. The Council also suggested that a special effort be made to encourage smaller institutions to join in the development and effective use of scientific personnel.

Annual reports of the Director of the NHLI (NHLBI after 1976) to the President, as required by P.L. 92-423, record the efforts

of the Institute to reach the general goals set forth in the 1972 National Program. The reports emphasized the need to train personnel to fill vacant posts and replace losses from the pool of investigators, and the need to anticipate the future requirements of the community. They provided estimates of the magnitude of the problem in the several areas of concern. According to the first report,

> The Institute estimates that approximately 200 research fellows should be trained now (1972) in the blood research areas addressed by the National Programs.

Research areas with the largest number of unfilled positions were listed as the hemoglobinopathies, immunohematology, coagulation, and blood banking. In fact, the latter field was singled out for special consideration because of the urgency of the need for additional, adequately trained personnel. It was recognized that the number of training facilities was too few and that resources were inadequate for making effective use of newer techniques and knowledge emerging in this vital aspect of health care. The report did not distinguish, however, between specific research needs in blood banking institutions and the need for a special kind of blood bank professional capable of managing an increasingly complex organization. Such an organization would need personnel whose training provides the flexibility to adapt to new and sophisticated developments in the field.

Subsequent annual reports of the Director again focused on the plight of blood banking institutions and reiterated the plea for new trainees in the areas of shortage. In the Second Report of the Director (fiscal year 1974), approximately 140 research training positions in hematology were recommended; in the Third Report of the Director (fiscal year 1975), 240 positions were suggested, a figure later increased to 300. New personnel requirements were emerging not only as a result of advances being made in the science but also as a result of the addition, by congressional mandate, of new areas of responsibility to the activities of the DBDR.

Similar assessments of research training needs in hematology and blood banking appeared in the Fourth and Fifth Reports of the Director (fiscal years 1976 and 1977). "An accurate accounting of personnel availability and needs must be developed for both blood diseases and blood resources." Once again, a serious shortage of "qualified directors for blood banks and transfusion facilities" was noted. The latter publication reviewed several new training and development programs that had been added to the Institute's mix of support mechanisms. One having applicability to DBDR research training was the Minority Access to Research Careers (MARC) Program, which addressed the major problem of the lack of opportunities for individuals situated in academic circumstances

less favorable than others. Another was the young investigator research grant, now termed the new investigator research award, which the NHLBI began to use in 1976.

In the Sixth and Seventh Reports of the Director, which appeared in 1979, a decline in the overall number of Institute trainees and the persistent problem of recruiting and retaining investigators holding the MD were discussed. In response to the latter, another support mechanism, the clinical investigator award, was utilized in 1980.

Developments in the Division

As part of its 1976 Report to the DBDR Director, the Blood Diseases and Resources Advisory Committee addressed the problem of training in hematology and blood resources. The advisory committee considered the topic carefully, examined the various facets of training, and identified qualitative and quantitative needs in the areas of thrombosis and hemostasis, sickle cell disease, and blood banking. Recognizing the distinction between research training and clinical training, the advisory committee stated:

> Training for a career in research must be conceived broadly enough to allow for the inclusion of skills in the areas of clinical science, patient care, and teaching, for on the practical plane these skills are often inseparable from the presence of research objectives. We believe that even in the domain of research the concept of the broadest possible training should remain dominant, since training exclusively in specific areas is not likely to provide the breadth of experience and general insight which is essential to bring new approaches into a discipline.

General hematology also came under consideration. Among the ideas offered were that training should have continuity, that young physician investigators should have an opportunity for adequate research experiences, and that deterrents to candidacy for the MD and PhD degrees should be addressed. Special note was made of the acute need for "physicians trained for a career in transfusion therapy and capable of managing full-service blood banks and blood centers." In addition, "special consideration needs to be given to the training of PhD's" since "research in clinical as well as preclinical areas of hematology and blood banking increasingly involves those trained in the basic sciences."

The committee in 1976 also recognized the importance of quantitative estimates of immediate and future personnel needs, often discussed in terms of health care rather than research:

> It is recommended that discussion be undertaken with appropriate representatives of these clinical areas as well as with NIH with the objective of planning an integrated study of the human resources needed for related health care.
>
> Until further data become available concerning research and teaching needs in the area of hematology and blood resources, we consider a realistic goal for NIH training programs to be the graduation of about 40 academic hematologists.

And further:

> An adequate data base for determining numbers of traineeships in the various aspects of hematology is lacking. There is a need for a survey of academic manpower with respect to research, teaching, and patient care in the field of hematology.

The DBDR has utilized most of the mechanisms discussed above. In addition, because of a continuing belief that blood banking organizations were still unable to attract and train sufficient recruits for immediate and long-range needs, the Division announced, in 1980, the Special Emphasis Research Area (SERA) Program, which utilized existing training mechanisms but called specific attention to the research opportunities available in the blood banking sciences. Its objectives were to:

- Encourage qualified individuals to direct, or redirect, their research interests and investigative skills toward the blood transfusion sciences, thus creating a pool of highly qualified investigators with experience and skills in the disciplines needed to understand and explore effectively the blood transfusion sciences, and

- Provide support for qualified individuals to pursue a program of research in fundamental and clinical research disciplines related to the blood transfusion sciences and toward research in the logistic and administrative aspects of blood center and blood transfusion service operations.

The key requirements in this program were that an applicant receive training under the supervision of, or in association with, scientists knowledgeable in the blood banking disciplines and that the individual pursue research that is applicable to blood banking problems.

Developments in Other Organizations

Concern for the state of training in hematology and in the blood banking sciences has also been expressed outside the NIH. Professional organizations and the National Academy of Sciences have sponsored several major efforts to identify needs during the past decade. In addition, as previously noted, articles calling attention to the plight of that "endangered species," the clinical investigator, have been highlighted in the medical literature.

Ranney and others[1] published a survey of staff positions in academic hematology based on questionnaires circulated in 1971 to directors of most of the existing programs. Although the survey was informative, only minor aspects of the analysis dealt specifically with research needs or opportunities. Of the reported 1,159 academic positions in hematology, 10 percent were unfilled at the time of the survey. Approximately one-third of the time of the respondents was spent in research, of which 59 percent was identified as being in oncologic research, 25 percent in the red cell area, 19 percent in thrombosis and hemostasis, and 14 percent in immunology and blood banking. Only 40 hematologists were involved in blood banking research. The survey did not raise the question of qualitative and quantitative goals for hematologic research, either in the immediate or long-range future.

The Graduate Medical Education National Advisory Committee (GMENAC) issued a report in 1980,[2] an objective of which was to make a quantitative estimate of future needs. To a large extent, the committee ignored research training. The report was focused on clinical needs in hematology and oncology, in which a shortage of practitioners of approximately 10 percent was projected by 1990. Research needs were included in a category called "Other," without documentation. The overall methodology of this study has been criticized, and large gaps in data collection have been recognized.

Special concern for the training needs of blood bank personnel has been voiced in a number of focused studies. In each instance, as was the case in the 1976 report of the Blood Diseases and Resources Advisory Committee, the attention was directed to training blood bank managers rather than to training researchers who undertake scientific investigations in blood banks. For example, Shively and others[3] in 1971 surveyed medical directors and physicians of blood banks in over 2,000 organizations. Almost one-half of those who were contacted responded. Of the professional personnel involved, 88 percent were pathologists, only 3 percent of whom were involved full time. Very little time was devoted to research by any of these professionals. One result of the study was a recommendation to develop a program to train blood bank directors.

According to a 1974 report by Greenwalt,[4] "there is a need for at least 500 physicians in this field to meet service and patient needs." (Aster estimates that there is a need for at least 1,000 people in this category today.) The reasons for this deficit were varied. One related to the increasing sophistication of blood bank services, which created new demands on its personnel. Research requirements were not specifically mentioned. Another study was conducted in 1975 by an ad hoc committee on manpower development in blood banking sciences chaired by Aster.[5] This far-ranging report included projections of needs in all phases of blood banking, including the need for executive directors, administrators, recruiters, therapists, and technologists, and a specific request for an unspecified number of "scientific personnel" in blood banks.

Research Training in Hematology

The DBDR is the major national focus for research training in hematology. At the NIH, the Division supports the majority of training and career development activities in hematology (figure 1), and it is the sole supporter of research training in the blood transfusion sciences (blood bank research). The National Cancer Institute and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Dieases provide important support for trainees in the blood diseases area, but the amount of funding is less than that provided by the NHLBI (figure 2).

From 1972 to 1981, the DBDR almost quadrupled its research training and development support in regard to the dollars expended and more than doubled the number of positions funded (figure 2). The data presented in figure 1 indicate that in 1972, the Division supported 31 percent of all NIH extramural research training positions and dollars at the NIH devoted to training in hematology. Today, the Division supports 62 percent of the training positions and dollars. While the general trend shows a gradual increase in funding and in the number of positions supported by the NHLBI, the NIADDK, and the NCI, the distribution of funds and the relative positions of the first two of these Institutes shifted considerably between 1972 and 1974. At that time, the Division became the major supporter of research training and career development in blood and blood diseases at the NIH. Figure 3 and table 40 indicate the number of positions supported by the DBDR from 1972 to 1981 in each category of training and career development. Figure 4 displays the number of trainees in each of the four major program areas of the Division during the same period.

From 1972 to 1981, the three Institutes that share the major responsibility for blood research training and career development supported a total of 3,174 person-years at a cost of $64,418,000

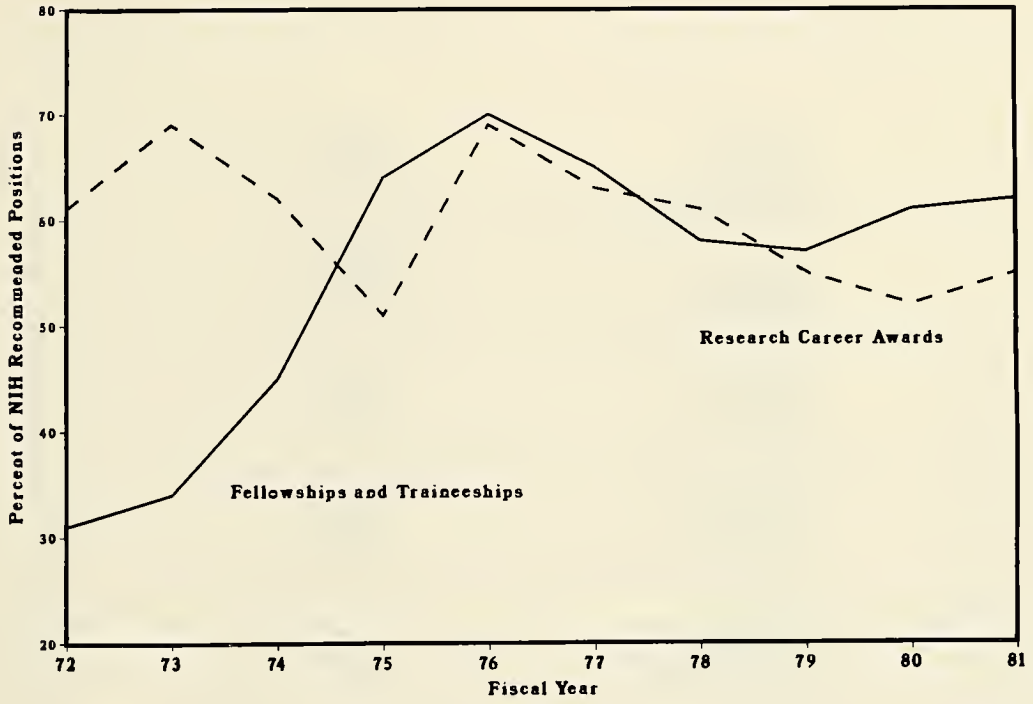


Figure 1. Research Training and Development Programs in Hematology
DBDR Positions Expressed as Percent of
Total Recommended Positions Supported by the NIH
1972 Through 1981

for an annual average cost per trainee of approximately $20,000. Since the average training period is 2 years, the NIH has provided approximately 1,600 graduates trained in blood research at an average cost of about $40,000 per graduate.

During the past 10 years, the research training supported by the Division has been in response to the following nine objectives.

Objective 1: Assure an adequate supply of well-trained investigators for research in blood diseases and resources by utilizing available predoctoral and postdoctoral support, such as the fellowships and training grants programs.

The Blood Diseases and Resources Advisory Committee, in its First Report to the DBDR Director in 1976, identified several areas in blood diseases and resources that had shortages of personnel. The committee estimated that a minimum of 180 positions

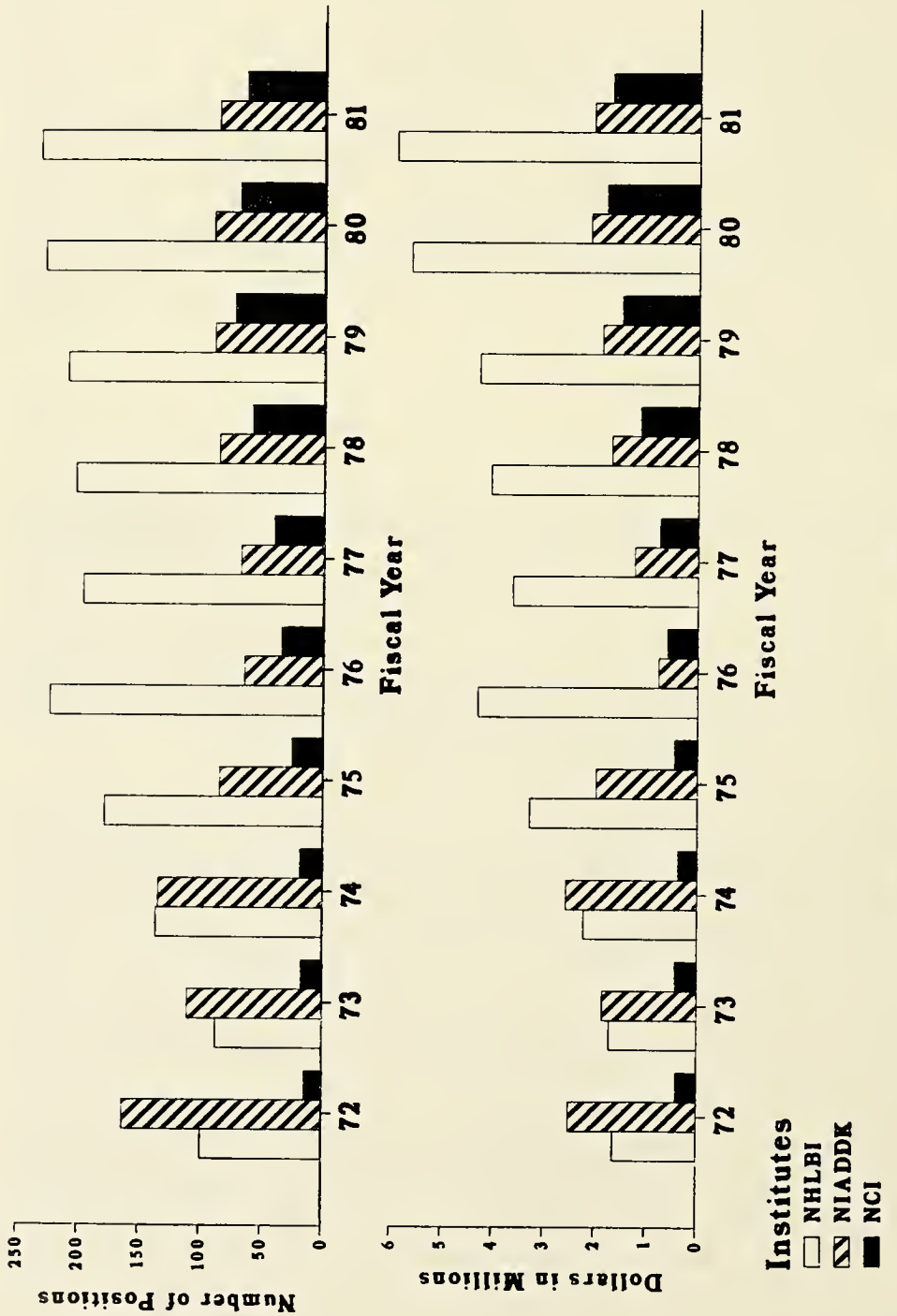


Figure 2. Research Training and Development Programs in Hematology
Expenditure and Number of Recommended Positions Supported by Three Institutes of the NIH
1972 Through 1981

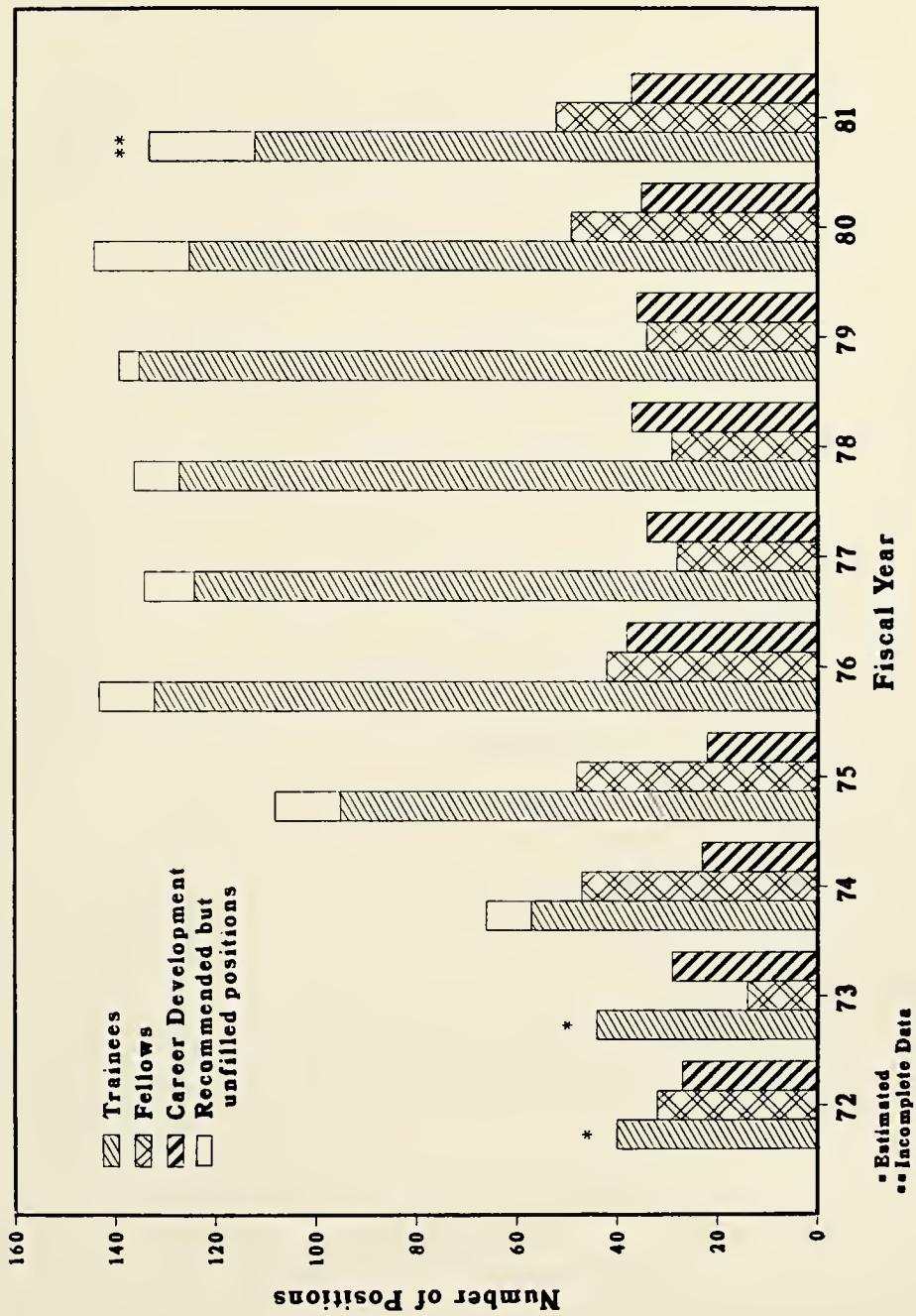


Figure 3. Research Training and Development Programs in Hematology
Fellowships, Traineeships, and Career Development Awards Supported by the DBDR
1972 Through 1981

Table 40. DBDR Research Training and Development Support in Hematology, 1972 to 1981

Year	Fellowships*		Traineeships*		Career Development**	
	No. of Positions	Average Cost Per Position in Thousands	No. of Awards	No. of Positions	Average Cost per Position in Thousands	No. of Awards	Average Cost per Position in Thousands
1972	32	10	10	40 est.	$16	27	$26
1973	14	10	11	44 est.	18	31	25
1974	47	13	13	28	32	23	25
1975	48	13	31	84	25	22	25
1976	42	15	38	121	22	38	25
1977	28	14	35	120	19	34	34
1978	29	14	39	127	18	37	36
1979	34	14	39	133	19	36	36
1980	49	20	37	125	27	35	36
1981	52	21	36	132***	24	37	39
Total years of support 1972-1981	375			954		320	
No. of people supported	287			734		70	
Average duration of training years	1.3			1.3		5.2	

*Designations of Fellowship (F32) and Traineeship (T32) are those in use since the NRSA was enacted. Previously called by other names. (See appendix A.)
**Includes K03, K04, K06, K08.
***Incomplete data.

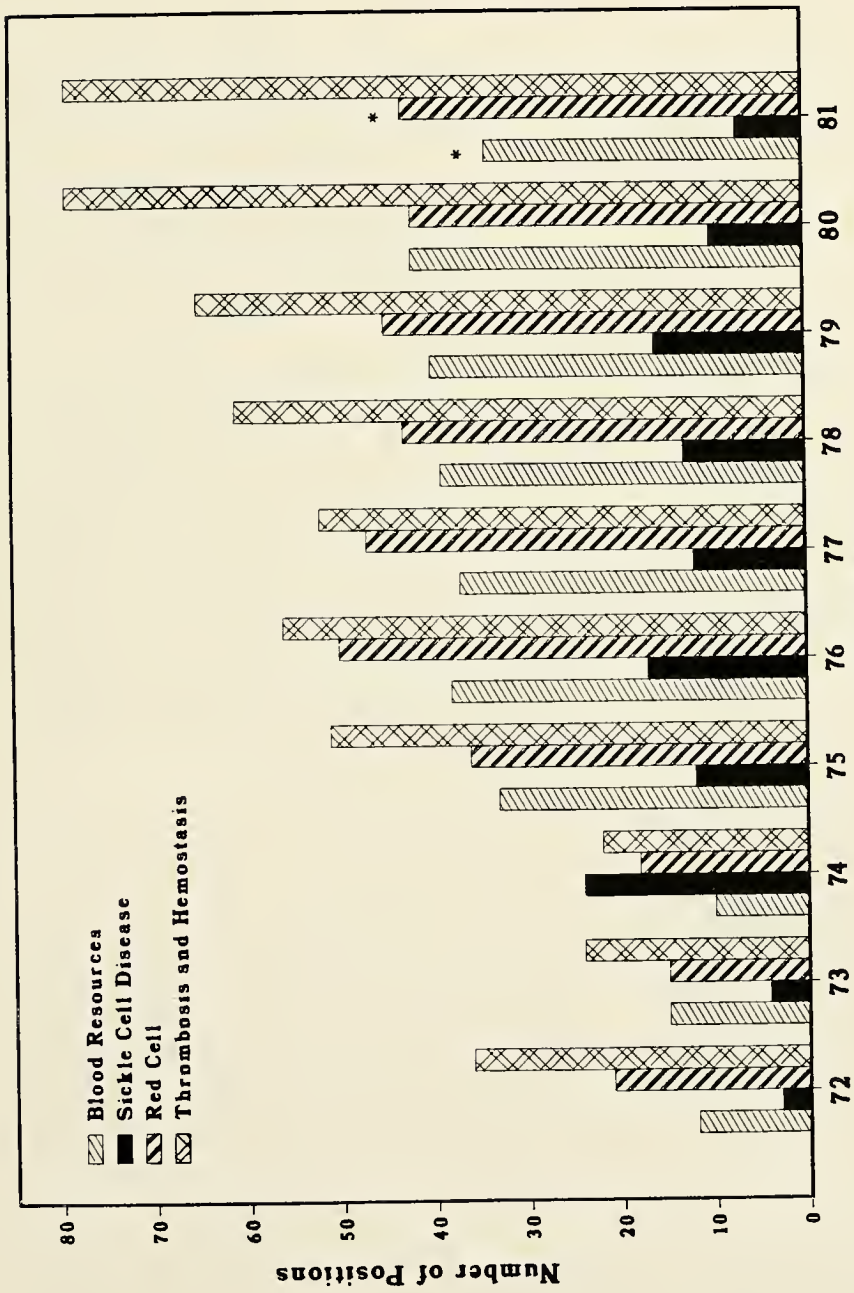


Figure 4. DBDR Research Fellowship and Training Programs
Number of Positions by Program Areas
1972 Through 1981

per year were needed to train investigators in these high priority areas. Two training support mechanisms (fellowships and training grants; after 1973, national research service awards: individual and institutional fellowships) were used to achieve the minimum goal set by the committee.

The success of the Division's research training recruitment effort is reflected in the increase in the number of positions from 72 in 1972 to 197 in 1981, an almost threefold increase in 10 years. During the decade, the Division supported a total of 1,329 positions (table 40). For the 6-year period between 1975 and 1980, DBDR data show that an average of 92 percent of recommended positions were filled. In 1972, only 2 percent of the Division's budget was committed to research training; in 1981, 6 percent was committed.

Figure 4 indicates the numbers of training positions from 1972 to 1981 in the four DBDR progammatic areas. For research in thrombosis and hemostasis and for research in the red cell area, the number of trainees increased over twofold. The number of training positions committed to sickle cell research remained stable, while the number in blood resources increased threefold. The latter reflects, in part, the growing complexity of blood banking, including its expansion into new disciplines. The expansion created a significant need to extend research training into additional areas. Between 1975 and 1980 (the period for which data are complete), 97 percent of the available training positions in the blood resources area were filled (231 of 239), and 68 percent (158) were held by MD's and MD-PhD's.

The mechanisms utilized to achieve these overall levels of training were:

Individual Fellowships. Over the 10-year period, 368 years of fellowship training were awarded to approximately 180 individuals. In 1972, 32 positions existed; in 1981, 52 positions were supported. Between 1975 and 1981, 78 fellows (individual) completed training under the National Research Service Awards Program. Of this number, 53 completed their training and 25 terminated their awards early. Of the latter, 15 did so to accept academic or industrial research positions, and 4 became funded by another Institute. Six terminated for financial, medical, or unknown reasons.

Sixty-two percent of these fellowships were pursued in the East and Northeast, 23 percent in the West and Midwest, 13 percent in the South, and 2 percent outside the United States. Seventy-nine percent of the awards were granted to males; 32 percent held the MD degree. Figure 5 shows the percent of MD's in training from 1975 to 1981. A small number, 18 percent, had received prior training as part of an

institutional training grant. At the time of their terminal report to the NIH, the 78 trainees had published 109 articles and 42 abstracts and had 73 papers in preparation.

Institutional Training Grants. The Division supported 1,097 trainees under institutional awards during the period 1972 to 1981, with the numbers increasing from 40 in 1972 to 143 in 1981. Detailed information on trainees and data for analysis are available only for the years 1975 through 1981. In this period, 42 institutional training grants in 34 institutions funded 505 trainees. An average of 12 trainees were enrolled per 5-year program. By December 31, 1981, 391 had completed the training period.

Characteristics of the group of trainees included the following: 78.6 percent were male; 75 percent had the MD or MD and PhD degrees (figure 5); 5 percent had other formal training experiences prior to accepting the award; and almost 6 percent went on to accept other training grants, new investigator awards, or career development awards supported by the

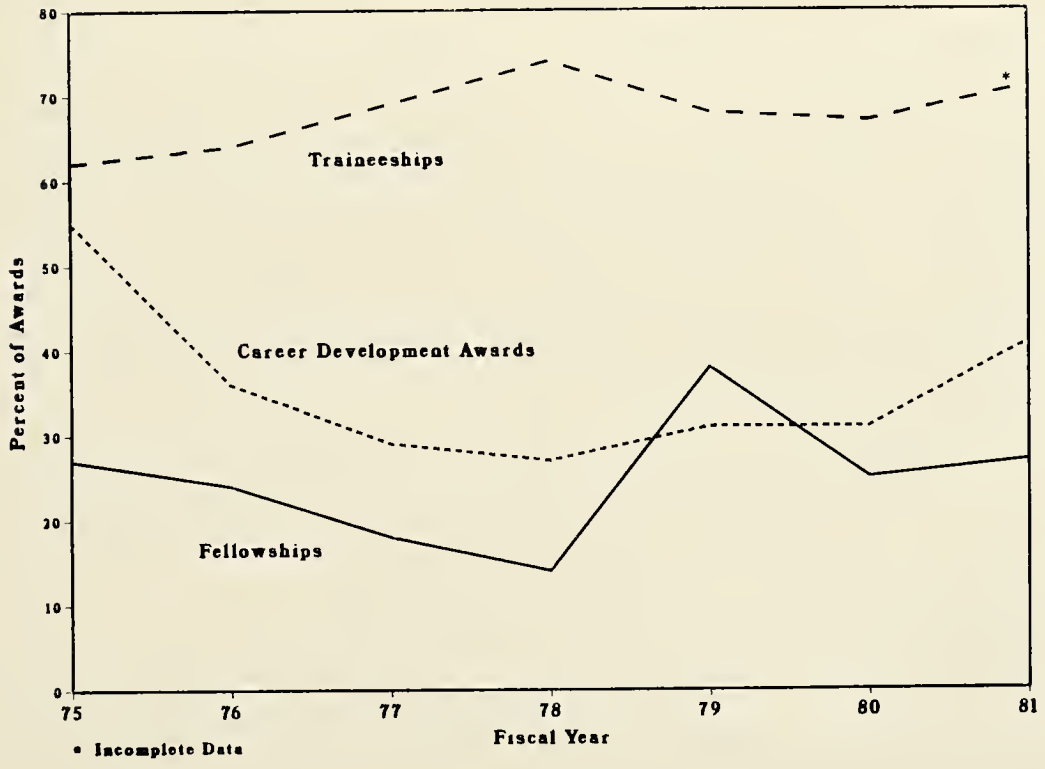


Figure 5. DBDR Training and Career Development Programs
Percent of Awards Held by MD's
1975 Through 1981

NIH or the Veterans Administration. Fifty-two, or 10.3 percent, failed to complete training, of whom 20 accepted an academic or industrial research position; 15 became funded by another Institute; and 17 left for a variety of other reasons, including medical and financial considerations and career decisions.

Publications reported by 355 of the 391 trainees completing their fellowships by December 1981 indicated that 247 had been involved in one or more publications and 11 had not. The remaining 97 made no mention of publications in their terminal reports to the NIH. The 247 individuals publishing articles accounted for 245 full-scale manuscripts and 156 abstracts, and they indicated that another 257 manuscripts were in preparation.

Objective 2: Facilitate changes in the direction of research careers toward the field of blood diseases and blood resources. Broaden the scientific background of experienced scientists through acquisition of new research capabilities in the blood diseases and resources area by using the special fellowship and the NRSA for senior fellows.

Between 1972 and 1976, the Division supported 15 special fellows in hematology. In 1980, the national research service award (senior fellow) program recreated the special fellow program, which had been terminated in 1973 when all Federal support for research training was temporarily withdrawn. During the 2 years of its existence, the Division has supported three senior fellows. These three individuals have the following interests: granulocyte preservation for transfusion purposes, genetic studies of beta-thalassemia, and platelet studies in von Willebrand's disease.

Objective 3: Accelerate the scientific development of investigators committed to research in blood diseases and blood resources by using the Research Career Development Program.

and

Objective 4: Continue earlier commitment to support highly productive, established investigators remaining under the Research Career Award Program.

From 1972 through 1981, 75 investigators were supported through the research career development awards (RCDA--KO3 and KO4) program. At the end of this period, 17 were still in training. Of the remaining 58 positions, 53 completed the award period while

5 terminated their awards early. These 58 investigators were supported for 307 award years, or an average of 5.3 years per award. In 1972, 24 positions existed; in 1981, 25 positions were supported. The latter number represents a decrease from the more than 30 RCDA positions supported by DBDR since 1976. The decrease, however, is more apparent than real, since a number of physicians chose to participate in the clinical investigator program that was established especially for physicians. Fifty-three percent of the awardees trained in academic institutions in the East and Northeast, 35 percent in the West and Midwest, and 12 percent in the South; 92 percent of the awards were granted to males; and 45 percent held the MD or MD-PhD degree(s). Figure 5 shows the percent MD's in all career development programs from 1975 to 1981. An impressive number, 79 percent (59 awardees), had received prior NIH training support; of these, 34 percent (20) had had 2 or more NIH fellowships or traineeships. Prior to receipt of the RCDA, 59 percent (44) were already principal investigators of NIH grants. Only 3 of the 58 graduates between 1972 and 1981 (including 5 who terminated early) no longer appear to be in research.

A 10-year followup of 24 individuals who received the RCDA in 1972 shows that 21 remained in research after completing their training. Of these 21 awardees, 19 received 2 or more NIH grants; 10 became directors of training grants; 6 were principal investigators of program projects; and 1 is principal investigator of a Specialized Center of Research (SCOR) in thrombosis. Twenty conduct research in the same area that was pursued under the RCDA, and 1 now conducts research in nutritional deficiencies. The age of 18 of the RCDA recipients ranged from 30 to 40 at the time of their awards, with an average age of 35.6 years.

Research career awards support highly regarded, established investigators for the duration of their careers. Three ongoing awards were assigned to the newly launched DBDR in 1972. No awards have been made since 1964, when the program was terminated, but the commitments then existing continue to be honored.

Objective 5: Attract physicians to an academic research career by exposing talented students in health professional schools to a guided research training experience for up to 3 months during off-quarters or summer vacation (for example, NRSA for short-term training).

In fiscal year 1980, the Division awarded two grants under the national research service award for short-term training: students in health professional schools. These are 5-year awards that provide support for 56 talented students for up to 3 months. The Division will fund these two grants through fiscal year 1985.

Objective 6: Encourage newly trained physicians to develop clinical and basic research interests and skills in blood diseases and blood resources by programs such as the Clinical Investigator Award Programs.

The Clinical Investigator Award Program was initiated by the National Heart, Lung, and Blood Advisory Council in fiscal year 1980 in order to attract young physicians to careers in clinical investigations. In fiscal year 1980, the DBDR supported two clinical investigator awards for training in thrombosis and hemostasis research, and in fiscal year 1981, seven additional grants were awarded. The disciplines for these recent recipients are thrombosis and hemostasis (three positions), sickle cell disease (two positions), and blood resources (two positions). This program is being monitored to determine its effectiveness in achieving its objective.

Objective 7: Continue characterization and analysis of the research personnel pool in blood diseases and resources to determine if present personnel needs are being satisfied and how future needs can be met.

and

Objective 8: Evaluate the Division's research training programs to determine their responsiveness to present and projected research needs.

In 1978, the Division of Blood Diseases and Resources, the Division of Heart and Vascular Diseases, and the Office of Program Planning and Evaluation inaugurated a 2-year pilot evaluation to acquire a blood diseases and resources and hypertension research manpower data base. In fiscal year 1980, the study was expanded to include blood investigators supported by the NIADDK and by the NCI during fiscal year 1978. The enlarged base for data collection included most of the NIH-supported blood researchers funded during 1978.

The purpose of the evaluation project was to establish the characteristics of successful investigators funded in fiscal year 1978. In addition, an attempt was made to include past NIH-supported training and research interests. In blood and hypertension research, 1,130 principal investigators (recipients of grants and contracts) were asked to supply information about their professional careers and scientific interests. Approximately 91 percent of the blood researchers and 85 percent of the hypertension researchers receiving support from the NHLBI responded, and 79 percent of NIADDK-supported investigators and 68 percent of NCI-supported investigators in blood research responded.

A preliminary analysis of the data shows that information was received concerning 1,918 investigators conducting research in blood, of whom 778 were principal investigators and 1,140 associate investigators. The DBDR supported 52 percent (403) of all principal investigators conducting blood research in fiscal year 1978, the NCI supported 30 percent (231), and the NIADDK supported 18 percent (144). The majority of principal investigators were involved with only one research grant. Approximately 43 percent of the investigators working on DBDR-supported research in 1978 held the MD, MD and PhD, or DVM degree; 58 percent of investigators on NCI and NIADDK grants held such degrees.

Objective 9: Continue support of the Minority Access to Research Careers Program to broaden opportunities for participation of ethnic minority investigators in blood diseases and resources research.

During this period, one proposal was identified as appropriate for the programs of the DBDR and was supported through a reimbursement to the National Institute of General Medical Sciences.

Problems in Training Support

Serious concerns have been raised by program directors as well as by trainees in relation to training at the NIH. While some of these concerns reflect changing Federal policies with respect to support, others relate to changing needs and interests of investigators and to the emergence of new attitudes.

In the past, federal training programs have been characterized by "on-again-off-again" funding and by the short-term authority granted by the Congress. The 1974 act, for example, authorized the NRSA program for 1 year, and the 1975 act for 2 years. There was no authorizing legislation in 1977, and training was conducted under a continuing resolution. The 1978 act was for 3 years with no appropriation. Funds for fiscal year 1979 came from a continuing resolution at the level of fiscal year 1978 expenditures, and funds for fiscal years 1980 and 1981 were also from continuing resolutions. Late in 1981, the national research service awards were reauthorized for 2 additional years under the Omnibus Reconciliation Act of 1981. The budget for fiscal year 1982 is contained in a continuing resolution.

Other aspects of Federal training programs have also been discussed during the past few years, including levels of stipends for postdoctoral fellowships. They are considered by training

officials to be noncompetitive. In particular, they are considered to be too low to interest potential trainees and as such are out of touch with reality. While stipends have increased considerably since 1972, they still lag far behind increases in the cost of living. When greatly out of line, the low levels of the stipends make it difficult to recruit potential investigators, particularly those who hold the MD. For many physicians who are in the process of completing a residency training program, the levels represent a significant reduction in income. It is a cut that many young physicians with families are unwilling or unable to accept. Although the government has tried to address this problem by permitting the sponsoring institution to supplement stipends, sharply increased stipend levels are not likely, particularly during the present fiscal situation.

A second point of concern, which is expressed by trainees and trainers alike, involves the "payback provision." In this case also, a partial remedy has been offered recently by the Congress. Current directives eliminate the payback requirement for those in undergraduate training and for those obtaining only 1 year of training. Furthermore, some flexibility is allowed in meeting remaining requirements. Prior to the recent changes in the law, some directors of institutional training programs and individual trainees decried what they considered to be the unreasonableness of the payback requirement and believed that it was a major obstacle to attracting young investigators, particularly physicians, into the training cycle. While for many trainees the entire issue of the payback is a moot point at the present time, it seems likely that data will be forthcoming to enable analysts to examine the relationship between payback and recruitment, to determine how those involved have handled the payback requirement, and to base future decisions in this area on better information than has been available.

The Division's initial experiences with the payback provision are not consistent with the negative views that have been expressed. The small number of fellowship holders who finished training during the period of rigid interpretation of the payback provisions did not find it difficult to fulfill the requirement, and few fellows have reneged on their commitment. In the case of almost every one of the latter, ample justification existed to modify the requirement and no one is actually in default at this time. Supporting data for these impressions are found in the NIH Program Evaluation Report on the Status of Medical School Faculty and Clinical Research Manpower: 1968-1990, which reviews the overall picture of the payback requirement.

Data relating to the DBDR indicate that 51 of the 78 individuals receiving individual fellowship (F32) support since 1975 have completed or are completing the payback. Of this group, 68 percent were PhD's. Seventy-six percent of the 51 have done

so in academic or medical institutions, 12 percent in industry, 4 percent in government research, and 4 percent each in blood centers and research foundations. Fifty-seven percent completed the payback in an institution different from the one in which they received training, and a high number, 54 percent, continued to conduct research in the area of their training. The remaining 27 trainees have yet to complete their fellowships.

Data also indicate that of the 391 individuals who have completed training under institutional fellowship (T32) support since 1975, 211 have completed or are completing the payback. Seventy-five percent of this group were MD's. Of the 211, 90 percent have been or are in academic or medical institutions, 2 percent in industry, and 4 percent each in government research and blood centers. Because of medical reasons, only two failed to complete the payback. Forty-four percent (93 trainees) completed payback in an institution different from the one in which they received training, and a very high number (70 percent) continued to conduct research in the area of their training. The remaining 180 trainees have yet to complete their training programs.

Another concern is the number of traineeships available at the NIH in general and at the DBDR in particular. While the number of trainees increased in most of the years of the decade under review, a continuous increase was unlikely, particularly as the economy declined. The concept of stabilizing the number of training positions (and research grants as well) was fostered during the latter part of this period in an attempt to guarantee an adequate number of researchers in the next generation. While these numbers represent a definite reduction in terms of earlier projections, they seemed justified by the fact that fewer grant-support dollars were to be available in this period of double-digit inflation (which of itself would reduce the number of opportunities for newly trained scientists) and by the observation that many of the Institutes, and certainly the DBDR, were funding all or most of the technically meritorious candidates who applied for support. The latter situation probably reflects the difficulties in recruitment caused by such problems as the levels of stipends and the payback requirement.

Other reasons for a decline in the number of qualified applicants, particularly those with the MD degree, are probably related to forces outside the Federal support programs, including the lack of opportunity to enter a research track as a result of restrictions and limitations imposed by medical school curricula, by housestaff programs, and by requirements of specialty boards. According to the results of an Association of American Medical Colleges (AAMC) graduation questionnaire, the "perceived societal need for practitioners appears to be the strongest disincentive to research careers." In addition, many postdoctoral candidates have major financial needs and debts related to their education, and

when they are combined with the uncertainties of Federal training programs and declining academic opportunities, a career in research becomes even less enticing.

These considerations bear in particular on the serious problem caused by a reduction in the pool of physician investigators as reported in a number of publications during recent years. Intuitively, one understands the need to have suitably trained physicians manage clinical research investigations. Similarly, the perception exists that when dealing with medical problems, even at a basic research level, it is desirable to have doctors of medicine actively involved, if not leading, the efforts. While such perceptions may be difficult to support with firm data, many feel that the physician is better trained and better equipped than a holder of the PhD to deal with medical research questions. When patients are research subjects, few would dispute the validity of this point.

Data presented by Wyngaarden[6] tend to support the belief that the number of MD's applying for, and being awarded, research grants is decreasing at an alarming rate. A similar decrease in the number of MD's entering training or holding career development awards has also been claimed. The NIH program evaluation report indicates somewhat similar trends but provides other explanations for them. At the DBDR, the data are at variance with these conclusions. (See table 41 below.) The Division has experienced

Table 41. Research Training and Career Development Programs in Hematology

Percent MD's and MD-PhD's Supported by the DBDR, 1972 to 1981

Year	Total Fellows with identified Degrees	No. Fellows with MD and MD-PhD	Percent Fellows with MD and MD-PhD
1972	59	29	49
1973	45	27	60
1974	98	50	51
1975	154	84	55
1976	202	110	55
1977	182	101	56
1978	193	109	57
1979	203	115	57
1980	209	107	51
1981*	201	108	54

*T32 data incomplete

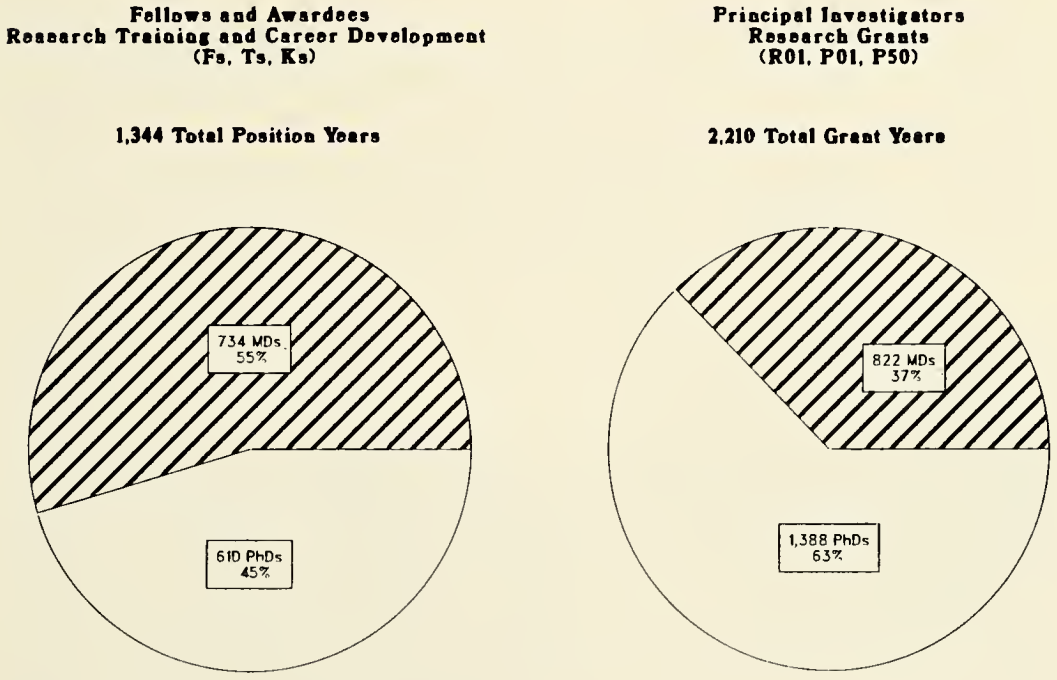


Figure 6. MD's and PhD's Supported by the DBDR
1975 Through 1981

few difficulties in recruiting MD's into training programs (figure 5). As noted in figure 6, 55 percent of the Division's trainees during the last 5 years held the MD degree, sometimes in combination with the PhD. During the same period, nearly 40 percent of the principal investigators in blood-related research were physicians. These percentages show only a slight drop during the 5-year period. As groups, the PhD's and the MD's were found to have comparable rates of success in getting their research efforts funded, but fewer MD's submitted applications for research support. Apparently, a significant number of MD's elected not to pursue a major commitment to research even after receiving 1 to 3 years of postdoctoral training. Thus, while there were only minor difficulties in attracting MD's into training programs, retention remains a problem that must be addressed if an active and expanding clinical research effort in hematology is to continue. Comparable data for each of the four major program areas of the DBDR are presented in figure 7.

In view of the experience of the DBDR, it is important to try to understand why the number of MD's involved in research in hematology did not decrease as was noted in other medical areas. Was this related to the fact that hematology is a specialty in

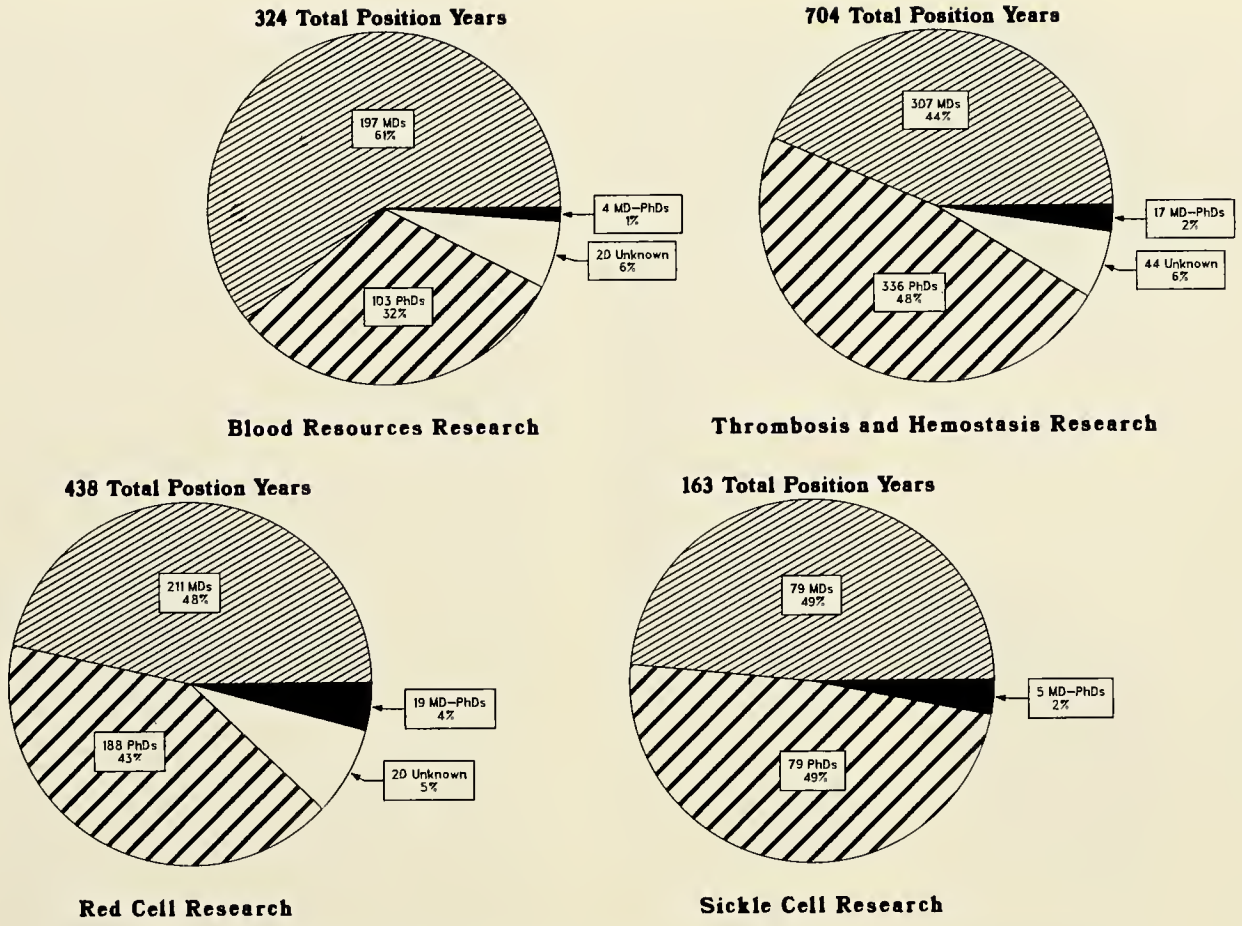


Figure 7. Research Training and Career Development Programs in Hematology
MD's and PhD's Supported by the DBDR in Four Major Research Areas
1972 Through 1981

which intensive laboratory activity attracts research-oriented physicians? Are hematologic research problems more easily pursued in a clinical setting than those of other specialties? Are the opportunities for both basic and clinical research in hematology greater than those in other disciplines? Are the numbers skewed by the inclusion of blood bank scientists involved in research? (See figure 7.) These, and similar questions, deserve future study to develop a better understanding of the motivations that lead researchers into careers in hematology.

Needs and Opportunities

Training programs that attract potential medical investigators to careers in basic and clinical research must offer recruits the opportunity to develop competencies in promising research areas. Moreover, they must have the flexibility to meet new and changing needs.

Medical schools represent a unique environment in which young people can be stimulated to pursue a research career, and laboratories with a hematologic orientation provide a milieu in which the interests of creative people can be directed into the field of blood disorders. The NIH and other organizations must provide medical schools and research laboratories with the intellectual and economic support that underlies successful training programs. Opportunities missed today will be translated into deficiencies of health care tomorrow, and a significant reduction in the number of recruits entering the training process can only result in a lack of capable investigators in the future.

Recruitment and Retention

As has already been described, training programs have evolved at the NIH in response to emerging needs. In spite of some instability in the programs, manifested particularly in the early 1970's, and despite a shrinking level of support, the Federal Government and the NIH remain committed to a strong effort. Graduate students and physicians contemplating a career in research are concerned, however, about limited opportunities for training and about the increasing difficulties in obtaining independent and ongoing grant support for research once training is completed. Such attitudes combine with other kinds of obstacles to discourage many from considering careers in biomedical research. Opportunities exist, however, for creative program development and innovative approaches to solving problems of motivation, entry, and retention.

As early as possible during their schooling, students should be exposed to the opportunities provided by an investigative career. At the high school and undergraduate levels, these efforts have largely been the province of the National Science Foundation and the National Institute of General Medical Sciences. Their support of training in basic disciplines should be encouraged and expanded, since without their efforts fewer candidates for more advanced training will be available.

The interests of medical students, unlike those of other students, need to be captured early in their professional education. Their particular needs suggest that more use be made of summer fellowships to help identify potential research talent, and that a new, combined program be considered that offers a year (or two 6-month training periods) at some convenient time during medical school, followed by a "guaranteed" year of postdoctoral research in an approved location of the trainee's choice. A second year of postdoctoral training might be included as an option. Such training would be somewhat comparable to the MD-PhD program for those who could not make the commitment prior to entering medical school or who were not accepted in one of the limited programs in existence.

Efforts to direct physicians into research careers should begin early in the clinical training period. A decision to pursue a research track involves complex issues, such as the availability of training positions, the level of stipend, requirements of clinical training programs, regulations of specialty boards, and availability of academic positions.

In the past, instability of authorizing and funding caused uncertainty about the future of training programs at the NIH; the shrinking budgetary allotment for training in the immediate future may increase this uncertainty. Opportunities for long-term stability of training programs can be enhanced by developing creative relationships with professional health groups and with private health-related organizations to supplement Federal programs and provide a "cushion" for a trainee when an interruption appears imminent. While none of the known hematologic or blood banking organizations appears to be in a position to take on a sizeable portion of such a burden, possible interactions may be worth examining.

Compensation for trainees with either the MD or PhD degree is of particular importance. By the time most physicians are able to enter a research track, they have acquired major financial debts and have family obligations that make it difficult to accept a stipend below what they have already attained as a result of their clinical training. For the PhD, it is difficult to resist the lure of industry, which, in increasing numbers, siphons off the

best talent by offering salaries far in excess of what a fellowship carries. Of 25 fellows who did not complete their NRSA training, 20 percent withdrew to accept industrial positions.

These considerations make it necessary to examine carefully the Federal regulations concerning stipends. In view of the fact that the government is unlikely to raise these stipends in the present economic situation, medical organizations, research institutions, and industry may need to make a greater effort to augment salaries and to support training opportunities so as to ensure an adequate supply of scientists for the future. The alternative of funding fewer trainees at higher stipends is incompatible with the need for additional trained investigators in the field.

The requirements of clinical training programs and of specialty boards, which fall outside the scope of the NIH, tend to inhibit a physician from considering a research career. The period of clinical training for board qualification and certification is often too long for the individual who is going into academic medicine. Several suggestions have been offered to improve this situation. One of the residency years might be replaced by a clinical research fellowship year. The overall preparation time would be shortened for the clinician, who needs at least 3 years of research training in order to do significant research.

In general, efforts should be made early to interest young physicians in research careers. Trainees who complete clinical training before they start their research training are experienced clinicians and nascent investigators. They approach their research with uncertainty and may experience frustrations that divert them into clinical medicine for the rest of their careers. A program in which trainees take 2 years of research training after the first year of residency and return to complete the remaining years of residency might offer an attractive alternative. This kind of approach was used in early NIH programs, in which many of today's academicians were trained. After completing internships, individuals came to the NIH for 2 to 3 years and then returned to their sponsoring institutions for further clinical training. A similar program is in place in several universities at the present time.

A variation of this theme is a "student year-out" during medical school with subsequent short-term research experience when possible, in a program that would be designed to interest the young scientist early in his career, as previously described. Another variation is an NIH program that would provide the 2 years of training, also described above, after the first year of residency, which would be followed by an additional 1 or 2 years of development upon the completion of clinical training. The latter 2 years of support would be in association with limited

research funding. Such a program would be similar to the clinical investigator award but would come earlier in the career of the beginning researcher.

This approach to programming would reduce uncertainties and delays and would ease the burden and risk placed on the young trainee and nascent investigator. With present programs, it is difficult for biomedical scientists to plan an academic career, for long periods can occur between opportunities to obtain support. A mechanism to ensure that continued funding goes only to people making satisfactory progress would, of course, be necessary.

Considerable discussion has taken place about the relation between MD and PhD investigators. The fact that two classes of investigators exist has caused confusion. There are PhD's who are pursuing basic research, and there are MD's who are pursuing what is considered basic research. The MD's in research fields need to be as well trained as the PhD's in order to compete for funds in today's market. It is crucial that clinical departments recognize that to compete, these young individuals need adequate time for research. Departments of medicine are therefore likely to develop arrangements in which they have clinicians who do little more than clinical medicine with some activities that might be called clinical investigation, and a class of individuals, composed of well-trained MD's and PhD's, who are pursuing basic research. The successful research group in a clinical department will be one that has time for research, has expertise in the latest techniques, and integrates MD's and PhD's in a collaborated pursuit of knowledge.

Stability in Research Grant Support

Present day fiscal realities make it unlikely that the Federal research budgets will increase at a rate greater than, or equal to, that of inflation. The negative aspects of not competing successfully for grant support and the demoralizing and depressing consequences of failure to get funded are surely major factors in keeping talented scientists away from the investigative laboratory. Recognizing the special needs of those competing for their first grant, the NIH has developed the new investigator research award. After 3 years, a "new investigator" competes with other scientists for traditional grant support. This may be an area for imaginative program development by the NIH. The role of the private sector, working in concert with the NIH, should be explored for help in funding research that is meritorious but not fundable through the NIH. For example, the philanthropic health organizations that are already engaged in direct or indirect support of biomedical research might extend support of peer-reviewed

applications that are submitted to the NIH by assuming responsibility for the ones that the NIH cannot fund.

An approach that the NIH might consider is to modify the clinical investigator award to provide research support beyond the 5-year period of salary commitment. A similar approach would be to associate research support with either the RCDA or the senior research fellowship award so that, in the former, concurrent with the salary stipend, and, in the latter, overlapping with the traineeship, research funds are made available. Since the NIH has in the past been reluctant to superimpose substantial research support upon a salary award, trainees have wasted valuable time by repeatedly submitting applications until they receive support that is adequate for pursuing an academic career.

Program Evaluation — Quality Control

As science has become more specialized, more complicated, and more technical, the difficulties in achieving adequate training in a reasonable period of time have multiplied. While the location of a training site is rarely of concern, the quality of the educational experience is of profound importance to the trainee. Unfortunately, the applicant usually does not have enough information for making reasonable judgments about the "quality of training." Yet one wonders whether there are ways to evaluate this quality. If one were to analyze the structure and function of the programs that have become continuing sources of skilled and productive investigators, can the elements that are critical in such successes be identified? Would such information lead other institutions to modify their own training programs?

It is through the institutional training grant mechanism that the NIH puts its trust in specific program directors and asks them to select the most promising candidates, some of whom may become the leaders of the future. It may be easier to assure quality here than in cases of individual fellowship grants, where an evaluation of the training environment may be very difficult. Should greater emphasis, therefore, be placed on the former, and should the training environment for the individual research fellow meet specified criteria for assuring a balanced exposure to both basic and clinical research, so that the experience will be sufficiently broad and adequately preparatory for a productive research career?

One way of evaluating NIH training activities is to examine the outcomes of current programs. This kind of effort has been undertaken recently by the NHLBI. The retrospective data reviewed earlier dealt with a limited range of questions. The information, however, does not evaluate need, quality, or effectiveness of programs. Only limited information is available on the publications,

funding, and academic records of those who have completed training. Prospective data collection, using modern techniques of inquiry and long-term followup, may help provide the kind of information needed to make a comparative evaluation of the training mechanisms used by the DBDR and to assess the capability of trainees and the quality of the environment where they receive their training.

Requirements of Blood Banks

Part of the difficulty in addressing the training needs of blood banks stems from a lack of a clear definition of what it is that the NIH should support in blood bank research. With a number of important exceptions, blood banks have not been institutions where primary research is conducted. Nevertheless, blood banks represent a valuable resource, and they have a potential for investigative activity that has not been utilized. Blood-bank-related research should be undertaken by collaborative groups involving immunologists, hematologists, and pathologists. Programs to train such individuals already exist in the DBDR.

The management of a blood bank, on the other hand, involves skills in preparing and delivering a safe and effective product. Support for the training of such managers is largely the responsibility of the various professional organizations of blood banks. Blood bank managers and their boards of directors, however, must become knowledgeable in the role of research in the transfusion area. They should develop an interest in supporting such activities even if, on the surface, the activities involve the apparent utilization of scarce dollars that are unlikely to be recouped from third party payers. Even more important is the need to enhance the credibility of the blood bank as a suitable setting for scientific research. A suggested mechanism to meet this need is an academic award in transfusion medicine.

Transfusion medicine is 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). At least five directions are involved in the full development of this medical area: basic research leading to a better understanding of the normal physiological function of diverse blood elements and how deficiences and excesses of these substances may result in clinical disorders; clinical research on how best to utilize transfusion medicine in the treatment or prevention of specific disease states; applied research to develop better methods in the collection, isolation, and preservation of cellular and soluble blood elements; development of techniques that will increase the safety of transfusion procedures; and scientifically based studies of the logistic, economic, social,

and behavioral aspects of the collection of blood and blood components from donors and the distribution to user facilities.

Future Personnel Needs

Research personnel needs are difficult to quantify. Nonetheless, medical planners find it advisable to estimate training requirements in order to make projections for the future. Such projections must be approached with caution because, even in the relatively short-term future, they are fraught with major uncertainties as technical needs and scientific modes shift and as scientists change disciplines and bases of operation. Furthermore, wastage will occur. Some trainees do not progress to compete successfully for advanced training programs. If as many as 50 percent of the graduates of the training programs, however, advance to significant positions in academic medicine, the investment is overwhelmingly profitable.

In the absence of specific quantitative guidelines to estimate the need for training positions, how can this problem be approached? For support of research training in hematology at the NIH, a simplistic answer might be that an estimate of need can be linked to the ratio of blood-disease-related research at the NIH to the total extramural research budget, which is, roughly, about 5 percent (in fiscal year 1980, $180 million: $3.6 billion). Thus, of the 10,000 trainees supported by the NIH at any time, about 500 per year should be identified as hematologic. Since the DBDR supports about two-thirds of all trainees related to hematology and oncology (three-quarters of all nononcological trainees), the Division should support about 350 people per year in this category.

Although future trends for research are hard to predict, and what seems remote and uninteresting today may suddenly erupt into a fascinating field of inquiry tomorrow, are there ways to develop more accurate and meaningful projections? How can the data be accumulated on which to base such projections? What kinds of data are desirable? Can appropriate models be built that would allow one to develop algorithms for projecting these needs? Once again, how can research needs be separated from demands of a clinical specialty area? Should an attempt to separate be made?

It is likely that models can be devised that are suited to reasonable projections. Efforts to examine such prospective methods should be undertaken involving, for example, the collection of data that take mobility of personnel into account (such as the number entering the system, the number dropping out, career duration, and interfield moves) and opportunities to follow an investigative career (such as positions available, funding opportunities, and availability of resource).

Finally, who should be involved in pursuing these efforts? Separate committees sponsored by the NIH and by professional groups have provided guidance in the past, and organizations devoted to medical specialties have pursued their individual goals. It may be appropriate for the NIH and such organizations to collaborate in monitoring developments in the field of research training and in trying to anticipate future needs of the specialty. Such a group, including representatives from the American Society of Hematology, various blood bank organizations, and blood-related health foundations as well as individuals familiar with training at the predoctoral and postdoctoral levels, should provide a meaningful forum for those concerned with these problems. In any such group, a specific effort should be made to include minority representation as well as to involve the smaller and less visible organizations related to the fields of hematology and blood banking. Candidates for the PhD and medical students should also have an opportunity for participation by whatever mechanism appears functional and reasonable. Such a concerted, organized effort may help guide the field of hematologic research.

Specific Needs in the Division's Program Areas

Specific needs in the DBDR program areas cannot be presented in quantitative terms, but several general statements can be made.

- The increasing pace and complexity of research has not been accompanied by a concomitant increase in the number of trainees in several areas of investigation. One such example is seen in sickle cell disease research. (See figure 4.)

- New concepts are emerging that call for investigators in disciplines previously not emphasized. Just as the 1970's demonstrated a need for scientists trained in nucleic acid chemistry, gene manipulation, and cloning technologies, the 1980's may require a cadre of investigators conversant in bioengineering and surface phenomena as manifest in cell-cell interactions and rheology.

- Discoveries are being made that link known disorders with each other. Pursuing such associations will require investigators with multidisciplinary training. Some red cell metabolic disturbances, for example, relate to neurologic disorders, and the many drug and environmental reactions with hematopoietic cells require an understanding of both pharmacology and pharmacogenetics.

- In certain areas, only a limited number of investigators of long-standing reputation are active. Efforts to train new researchers and to interest others in the subject

matter should be encouraged. An example of such a need is seen in the area of red cell enzymes where the same small group of scientists has been active over a number of years.

- Some important areas of expertise have not yet made a major contribution to hematology. Epidemiology and biostatistics, for example, have had only a limited impact on the field, but there are ample opportunities for creative and imaginative research using these tools. Similarly, organic synthetic chemistry, as related to the perfluorochemicals and oxygen chelator chemistry, has not attracted many hematologic investigators, but the subject offers many opportunities for research.

Areas in which there are not enough trained scientists are:

Red Cell - Sickle Cell Disease

Metabolism - enzymology
Genetics - recombinant technology, protein synthesis
Bioengineering - surface phenomena, rheology

Thrombosis and Hemostasis

Structure-function relationships - protein synthesis, DNA technology
Immunology - monoclonal antibodies
Physiology - cell biology
Complement - function
Bioengineering - rheology

Blood Resources

Transfusion medicine - basic and clinical aspects.

Recommendations

- Improve recruitment and retention of potential investigators by:

  - Early identification

  - Developing new programs early in the formal training process

  - Initiating innovative opportunities for further training and research support in the postdoctoral period.

- Collect data on trainees prospectively for a comparative evaluation of training mechanisms and for an assessment of capabilities of trainees and of the quality of the environment of their training.

- Consider new directed programs to encourage the development of the blood transfusion sciences for the special needs of blood banks. A transfusion medicine academic award may be appropriate for this purpose.

- Explore creative relationships between the private and public sources of training support so as to improve the support available and to encourage a greater degree of stability in the various training programs.

- Consider forming an overarching organization that brings together all interests in hematologic training.

Appendix A

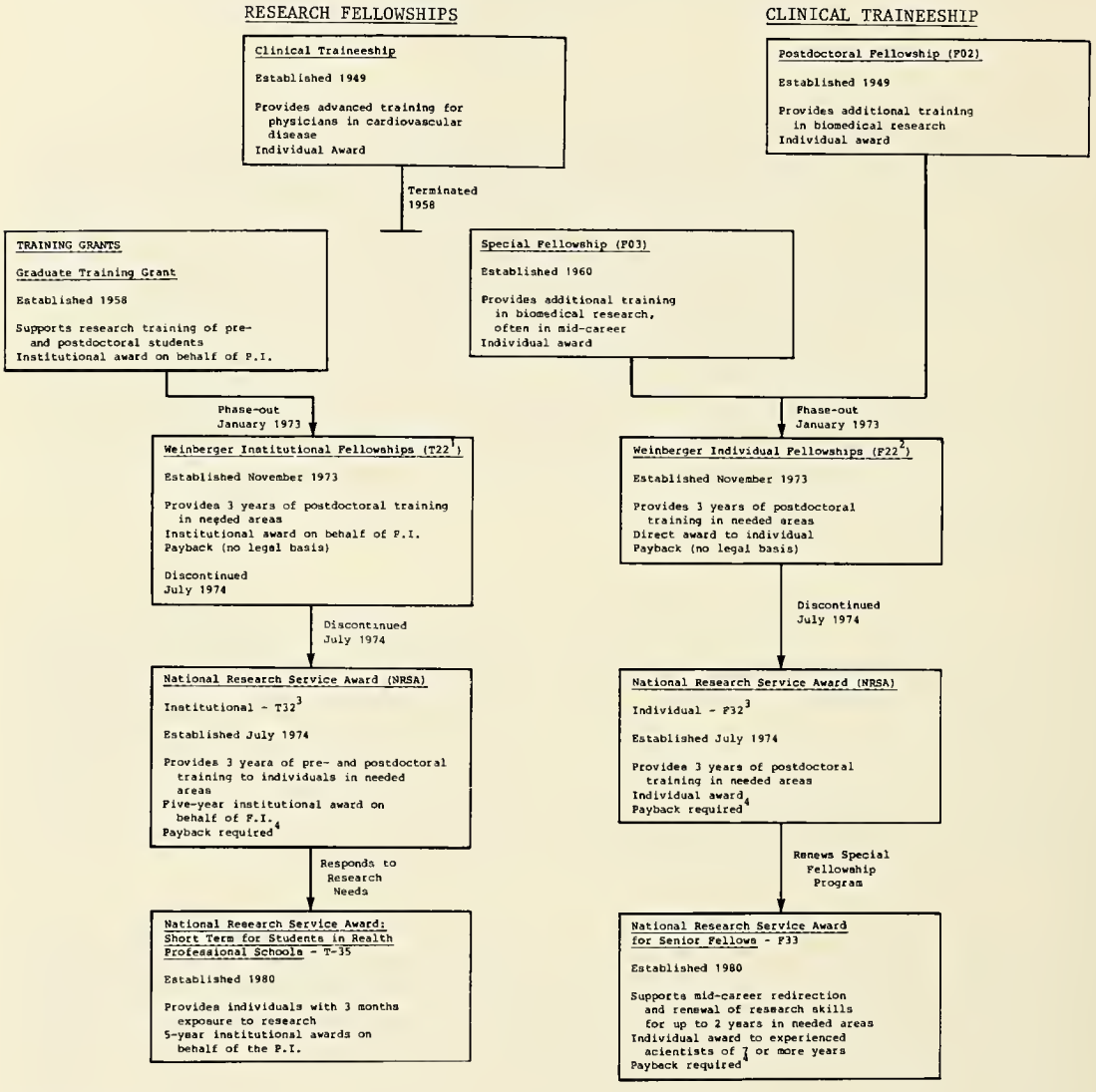
Legislative and Programmatic Background

The Ransdell Act (P.L. 71-251) of 1930 established the National Institute of Health and created a system of fellowships for duty at the NIH and at other medical and research institutions. The National Cancer Act of 1937 (P.L. 75-244) established the National Cancer Institute, the first of 10 separate categorical health-oriented Institutes of the NIH, and initiated the Federal Government's first major fellowship program. A few years later, the Public Health Service Act of 1944 revised and consolidated laws pertaining to PHS and gave NIH additional broad legislative authorization for fellowships and training. Expansion of the NIH into additional disease-oriented fields began in 1948 with the establishment of the National Heart Institute (NHI) and the National Institute of Dental Research. Other Institutes followed.

In the immediate post-World War II era, it was recognized that a wholly inadequate base of scientifically trained personnel existed in nearly every field of medicine. This recognition led to major efforts on the part of the NIH to develop programs capable of training a larger number of biomedical scientists in the basic sciences. At the same time, these efforts also had the objective of creating a core of faculty members, trained in the clinical specialties, to help meet the anticipated needs for teaching, research, and service in these areas.

During the period 1937 to 1946, the NCI awarded individual training grants in two areas: postdoctoral research fellows and clinical traineeships for physicians to improve their capability in diagnosis and therapy. In 1946, the NCI expanded its program to encompass predoctoral research fellowships. As the other Institutes came into being, each initiated its own training effort based, in part, on the precedents established by the NCI. (See figure 8 for the evolution of NIH training and fellowship programs.)

The first departure from the pattern of awards to institutions on behalf of individuals occurred in 1948 with the initiation of undergraduate training grants. Rather than provide stipends for individuals, these awards sought to strengthen the environment in which the categorical interests of cancer, heart, and mental health were pursued. In 1950, the NHI modified this training grant mechanism for use at the graduate level and included funds for training stipends. From this emerged the graduate training grant, which soon became a major NIH instrument



[1] DHEW decision to phase out T01 programs. Applications reviewed by Council 6/74 converted to T32.

[2] DHEW decision to phase out F02 and F03 programs. Applications received 9/73 and those received by Council 11/73 and 3/74 converted to F22.

[3] The National Research Act (P.L. 93-348) abolished all previous research training authorities.

[4] Revised 1981. Payback waived for first year.

Figure 8. Evolution of Research Training and Fellowship Programs at the NIH 1949 to Present

of training support. Also at this time, the desire for training to meet faculty and research needs, rather than to satisfy the requirement for specialty clinical training, became paramount. As a consequence, most training programs without such capability were phased out over the next few years and the importance of clinical training under NIH sponsorship was diminished.

Part-time fellowships, usually for summer work, were initiated in 1954 for medical and dental students. This program was intended to stimulate student interest in research, permit early identification of research talent, and expose selected individuals to a research experience. In 1957, a program to permit medical and dental students to spend a year in research between their preclinical and clinical years was established. In the same year, an experimental program was initiated in which medical schools were encouraged to develop new approaches to the identification, selection, and training of medical students for academic careers.

By the late 1950's, broad authorities for training efforts had been enacted, an array of support devices developed and tested, and a review and selection system established that was based on assessment of merit in national competition by peer judgment. Thus, the NIH was prepared for the circumstances that required a large-scale expansion of the NIH training effort: growing demand for scientists and teachers, an adequate pool of potential trainees, and the imminent growth and extension of the biomedical research effort as the consequence of a national consensus.

The establishment of general research support (GRS) grants, authorized under P.L. 86-98 in 1960, made available to medical institutions flexible funds for use at the grantee institution's discretion. Portions of these awards were used for student stipends which allowed the NIH to discontinue the postsophomore and part-time student fellowships. However, with the discontinuation of GRS in the early 1970's, the NIH found it necessary to reestablish, in 1980, a short-term award (under the NRSA) to provide a maximum 3-month structured research training experience for students in health professional schools.

Decades ago, the NIH perceived that the continued strength of universities and research institutes depended upon creating a stable basic structure composed of the best minds the institutions could attract. To assist in achieving this end, the Research Career Award Program was initiated in 1961. This program incorporated the senior research fellowship programs previously undertaken by several of the Institutes. It had two levels: research career development awards, which were granted to promising young scientists in the beginning stages of their careers; and research

career awards, which were granted to fully established scientists to enable them to devote maximum time to their research activities. In 1964, the NIH began to phase out the latter program when it was determined that NIH goals would not be advanced significantly. The research career development award, however, has flourished over the years. Its evolution is traced in figure 9.

Fellowships and research training grants continued to evolve to meet changing research training needs as the health sciences progressed. Constant support by the scientific community and the Congress kept the NIH research training program more or less in partnership with research programs until January 1973, when an administrative decision was made that phased out all training and fellowship programs. It was claimed that the NIH already had met most training goals and any remaining needs could be met most effectively by the private sector. The administration held the philosophy that demand would determine supply. The scientific community vigorously protested this decision, however, with the result that a new program of individual and institutional fellowships, informally designated the "Weinberger Program," was announced in November 1973 by Mr. Caspar Weinberger, then Secretary of HEW. This program was short-lived, since in July 1974 Congress enacted the National Research Service Act of 1974, which abolished all previous training authority and substituted individual and institutional national research service awards (NRSA). In 1980, the NRSA for senior fellows was reestablished by the NIH to substitute for the "special fellowship," which was abolished with the passage of the NRSA act of 1974.

To bridge the transition from training status to that of established investigator, a young investigator research grant (YIRG) was developed in the mid-1970's and used on a pilot basis by the NHLBI. Later, the program was adopted by the NIH, and its name was changed to new investigator research award (NIRA). The program provides funds for relatively inexperienced investigators with meritorious research ideas.

Seven NIH Institutes support clinical investigator awards, which recognize the special need to encourage physicians to commit themselves to careers in clinical and basic research in a categorical disease area. The National Heart, Lung, and Blood Advisory Council established its own clinical investigator award program in 1980 and made its first awards that year. Figure 9 summarizes this program.

To meet the special needs of minority scientists and institutions, the NIH initiated several programs to encourage ethnic and racial minorities to enter into and participate in the mainstream of biomedical research. They are the Minority Access to Research Careers, Minority Biomedical Support (MBS), Cooperative Minority

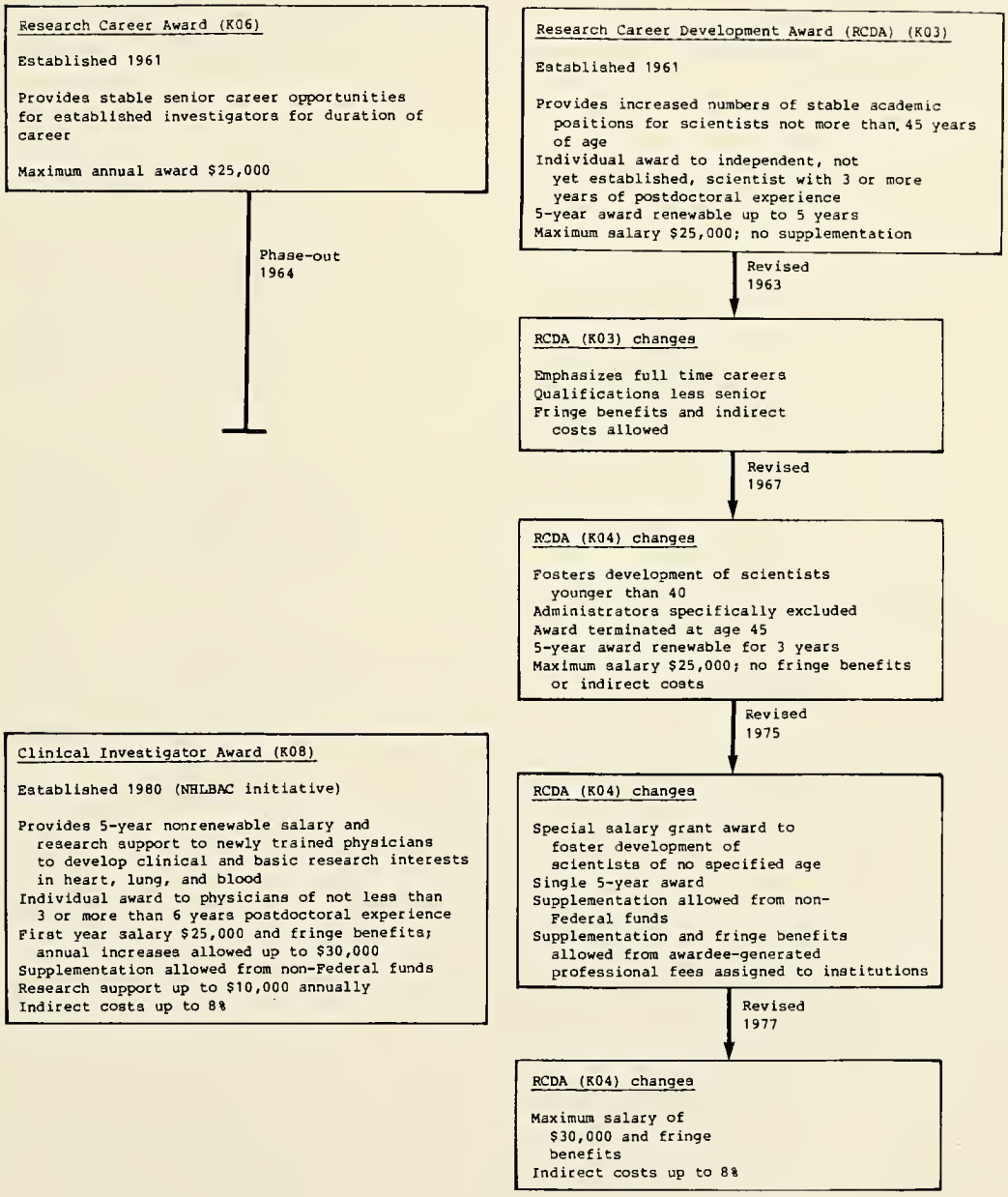


Figure 9. Evolution of the RCDA Programs at the NIH 1961 to Present

programs, and the extramural associates program. They serve to strengthen the research capability of minority educational institutions, motivate and prepare students for advanced studies, provide research training for faculty and graduate students, and familiarize minority and women's colleges with NIH-supported health science research in order to enhance their participation in NIH programs.

Appendix B

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National Heart, Lung, and Blood Institute

Volume 1. Progress and Promise
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Volume 2. Heart and Vascular Diseases
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Volume 3. Lung Diseases
NIH Publication No. 84-2358

Volume 4. Blood Diseases and Resources
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Volume 5. Companion Issues
NIH Publication No. 84-2360

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