THE JOURNAL OF
PEDIATRICS

FEBRUARY 1974 *Volume* 84 *Number* 2

MEDICAL PROGRESS

Blood transfusion: Merits of component therapy

*II. The clinical use of plasma and plasma components**

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BLOOD is often in short supply. By intelligent utilization of various cell fractions, several recipients may benefit from a single unit of blood, thus decreasing the over-all demand for this valuable substance. Just as there is seldom a need for all of the cellular components present in whole blood for replacement of one specific deficiency, there is similarly rarely a need for all of the substances present in the plasma to provide effective treatment of patients lacking specific plasma proteins. Since the advent of practical fractionation procedures, it is now possible to harvest and purify several plasma components including albumin, gamma globulin, fibrinogen, factor VIII, and factor II-VII-IX-X complex. This report reviews the use of plasma fractions in the management of patients with plasma protein deficiencies.

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**Part I appeared in the January, 1974, issue of the* JOURNAL.

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PLASMA AND PLASMA FRACTIONS

Plasma was first used on a large-scale basis during World War II. Since that time, technological advances in blood collection and fractionation have resulted in a variety of concentrated components of plasma for specific clinical uses. Plasma may be obtained during component preparation of packed red cells and platelet concentrate from fresh blood, from units of outdated bank blood, or by the technique of plasmapheresis. Fresh ACD or CPD plasma contains normal amounts of all

Abbreviations used
ACD: acid-citrate-dextrose
CPD: citrate-phosphate-dextrose
AHF: antihemophiiic factor
EACA: epsilon aminocaproic acid

coagulation factors and, if frozen within a few hours of collection, may be stored at −30° C. for up to 12 months with little deterioration in coagulation factors. Plasma obtained from outdated bank blood lacks significant amounts of factors V and VIII and should not be used in the treatment of these deficiencies; however, it may be used for replacement of factors I, II, VII, IX, X, XI, XII, and XIII which are relatively stable during 21 day blood

bank storage. In general, plasma stored at room temperature should not be used in the treatment of coagulation deficiencies because of the development of anticoagulant activity of fibrinogen degradation products.[115]

For many years plasma from a large number of donors was pooled with the objective of lowering the amount of anti-A and anti-B present, both by dilution and by neutralization of antibodies with soluble group A and B substances present in the plasma of donors who were secretors of those substances. However, such pooling often resulted in a product that still contained considerable amounts of anti-A and anti-B[116]; if infused into a non-group O recipient such pooled plasma was capable of causing a transient hemolytic anemia as well as cross-matching problems. This, coupled with the high risk of transmission of hepatitis,[117] has resulted in abandonment of the use of pooled random donor plasma.

Several techniques have been developed to harvest specific plasma protein fractions. The most widely used methods of plasma fractionation are based upon the use of cold alcohol precipitation procedures developed by Cohn and co-workers.[118] Fresh-frozen plasma is slowly thawed at 2 to 4° C. and centrifuged; a cold-insoluble protein is precipitated[119] which contains a considerable proportion of factor VIII and fibrinogen. After the cryoprecipitate has been removed, the supernate may be subjected to DEAE-Sephadex absorption which removes the factor II-VII-IX-X complex. By varying concentrations of alcohol, pH, temperature, protein concentration, and ionic strength, purified solutions of albumin and gamma globulin may also be harvested. Some of the purified fractions are useful in the treatment of coagulation deficiencies and make it possible to provide adequate replacement therapy without the infusion of large amounts of protein, which often produces circulatory overload in the recipient. Additionally the products can be tested for sterility, pyrogenicity, and potency; the last is especially important in the fractions used for coagulation deficiency replacement and in certain of the immunoglobulin preparations. Unfortunately, since fractionation procedures involve the use of large pools of plasma, hepatitis virus is potentially present in all components. Transmission of hepatitis does not seem to occur following the administration of gamma globulin preparations, either because the virus is removed during preparation or because antibody specific for the virus is present in the preparation. Albumin and plasma protein fraction U.S.P. are heat treated after fractionation to inactivate hepatitis virus and are not associated with hepatitis transmission. All other plasma components can transmit hepatitis, especially fibrinogen and the factor II-VII-IX-X complex. An undesirable effect of fractionation is denaturation of plasma proteins which may lead to recipient reaction[120] or may result in new antigenic determinants capable of eliciting host immune response after transfusion.[121]

Factor VIII replacement. The need for replacement therapy in patients with hemophilia A varies with the severity of the disease. Persons having from 5 to 25 per cent of normal factor VIII concentrations seldom suffer spontaneous hemorrhages and may require infusions of antihemophilic factor (AHF) only at times of environmental or surgical trauma. Persons with between 2 and 5 per cent of normal values only occasionally bleed spontaneously; it is only those with one per cent or less factor VIII who are subjected to frequent and spontaneous bruising, hematoma formation, external hemorrhage, and hemarthroses.

Many forms of replacement therapy are available for the patient with classic hemophilia. For many years the infusion of ABO-compatible fresh or fresh-frozen plasma was the only means of controlling bleeding, and circulatory overload often followed in patients infused with large volumes (more than 30 ml. per kilogram per day). The use of more concentrated preparations of factor VIII has dramatically diminished this problem, and at the present time even major surgical procedures may be performed on patients with severe hemophilia if factor VIII concentrations 25 to 30 per cent of normal are achieved.

A "unit" of factor VIII is arbitrarily defined as the amount of AHF activity present in 1 ml. of normal male plasma. Since the concentration of AHF in normal people may range from 50 to 200 per cent of a *mean* value, it is apparent that 1 ml. of plasma may contain from 0.5 to 2.0 "units" of AHF activity. The factor VIII activity of cryoprecipitate per gram of protein is 6 to 25 times that of fresh plasma, although only about one half of the total AHF present in fresh plasma is recovered. Cryoprecipitate may be administered without cross match and without regard for ABO group. Both cryoprecipitate and plasma are useful in the treatment of von Willebrand's disease, although therapy with plasma may be less expensive.

Other concentrated preparations of AHF are commercially available. Factor VIII rich fibrinogen prepared from Cohn fraction I provides concentrated factor VIII activity relative to plasma but contains more protein per unit of AHF than does cryoprecipitate. It also contains anti-A and anti-B, and transfusion of large amounts into non-group O recipients has led to red cell hemolysis.[122] Glycine-precipitated AHF is relatively free of other proteins; like factor VIII rich fibrinogen it

is reconstituted immediately prior to use. Other highly concentrated preparations are also available prepared by polyethylene glycol precipitation[123] or from glycine-precipitated polyethylene glycol fractionated cryoprecipitate.[124]

Patients with only minor bleeding may be effectively treated with cryoprecipitate or fresh-frozen plasma; major bleeding or surgery usually requires the use of one of the highly concentrated preparations commercially available. It is necessary to continue replacement therapy even after bleeding has stopped; patients with severe disease undergoing surgical procedures should have factor VIII concentrations maintained at from 25 to 30 per cent for 10 days and 10 to 15 per cent for the following 3 to 7 days.[123] Since a "unit" of AHF is defined as the activity in 1 ml. of fresh plasma, the number of units to give to achieve desired levels will be directly related to the plasma volume of the recipient (usually about 45 ml. per kilogram). One hundred per cent activity would correspond to a concentration of 45 units of factor VIII per kilogram of body weight. Factor VIII has a biological half-life of about 12 hours; about one fourth of an administered dose is distributed extravascularly.[125] Thus to ensure 25 per cent AHF concentrations at all times, at least 70 to 75 per cent concentrations should be achieved immediately after an administered dose if AHF is given early 12 hours (30 to 35 units per kilogram). The commercially available preparations offer an advantage over cryoprecipitate and plasma in that the number of units present is known; one may estimate from 70 to 100 units of AHF activity per bag of cryoprecipitate and from 200 to 250 units per bag of fresh-frozen plasma.

Inhibitors of factor VIII activity may be present in some patients and pose very difficult management problems. Large amounts of AHF may be necessary to neutralize inhibitors. A method for estimating the approximate amount of AHF needed has been described.[126] Treatment is not without risk, since the infusion of antigen may trigger a booster antibody response and result in even greater concentrations of inhibitor.

Treatment of bleeding episodes by the patient or family members at home has been shown to be of value. Prophylactic treatment programs have been shown to significantly decrease the morbidity rate of hemophilia A[127, 128]; the costs are great although they may be less than those which would result if no prophylaxis were given.[128] At present, it is estimated that only between 500,000 and 1,000,000 units of fresh-frozen plasma are harvested per year in the United States; prophylactic treatment of all hemophilic patients might require the use of over 10,000,000 units annually.[125]

Interestingly, Walsh and associates[130] reported that epsilon-aminocaproic acid (EACA) administered to patients undergoing dental extraction following a single preoperative dose of AHF resulted in no need for subsequent factor VIII replacement to control bleeding. Of 12 patients not given the drug, 7 required transfusion. Others have reported decreased need for AHF if EACA is used following surgical procedures.[131] Although still investigational, EACA adjunctive therapy may decrease the need for factor VIII replacement without compromising hemostasis.

Factor IX replacement. Factor IX is relatively stable in bank blood and plasma from outdated blood may be used as a source of the factor in the treatment of hemophilia B, as can fresh or fresh-frozen plasma. Factors II-VII-IX and X can be absorbed on DEAE-Sephadex columns and subsequently concentrated and used in the treatment of Christmas disease[129] or deficiencies of factors II, VII, or X.

Infusion of concentrated preparations of the II-VII-IX-X complex generally result in only 40 to 50 per cent in vivo recovery, possibly due to rapid extravascular diffusion or to determination of falsely high values of factor IX in the concentrate during in vitro assays.[129] Factor IX has a metabolic half-life of about 24 hours and concentrations of 25 per cent of normal will provide effective treatment of trauma and allow surgical procedures to be performed. Dosage and maintenance schedules of administration should be based on the half-life of the factor and the recipient plasma volume in a manner similar to that described for factor VIII replacement, but additional amounts must be given to compensate for the apparent greater extravascular loss (total dose of about 35 to 40 units per kilogram per day with the concentrated preparation).

In much the same manner that prophylactic AHF infusions have decreased the morbidity rate of patients with hemophilia A, prophylactic treatment of hemophilia B has also been shown to be effective.[129] The risk of hepatitis transmission with factor IX concentrate is high[132]; however, as techniques for detection of hepatitis B antigen become more sophisticated, it is anticipated that the risk of hepatitis will diminish. The II-VII-IX-X complex should not be used in patients with known liver disease or in situations where there is any suspicion of disseminated intravascular coagulation or fibrinolysis.

Factor I replacement. Fibrinogen has a half-life of 4 to 6 days; approximately one half of an administered dose enters the extravascular space and concentrations of from 50 to 100 mg. per 100 ml. of plasma are necessary for effective hemostasis (dose of 35 to 70 mg. per

kilogram every 4 to 6 days). Commercially available preparations can readily provide such concentrations; however, the risk of hepatitis is from 14 to 25 per cent. If commercial preparations are used, care should be taken to use material prepared from the same pool lot if repeated infusions are anticipated. Fresh or fresh-frozen plasma may be used effectively and carries significantly less risk of hepatitis transmission than commercial preparations. Since cryoprecipitate also contains considerable fibrinogen (about 250 mg. per bag) its use is preferred in patients in whom problems with potential hypervolemia are anticipated. Fibrinogen is often used in the treatment of obstetric emergencies such as placenta abruptio; it has also been used in the treatment of other forms of dissminated intravascular coagulation. Correction of the underlying disease causing the coagulopathy is probably preferable to the infusion of fibrinogen; however, if this cannot be done, heparinization of the patient[135] is probably preferable to the use of fibrinogen in controlling bleeding.

Replacement of other coagulation factors. Deficiencies of other coagulation factors occur relatively rarely and their discussion is beyond the scope of this review. Their treatment has been discussed by Johnson and associates.[125]

Albumin and plasma protein fraction U.S.P. Albumin infusion may be used to restore intravascular blood volume, to increase the amount of bilirubin removed during exchange transfusion of infants with erythroblastosis fetalis, and, occasionally, in the management of patients with severe hypoalbuminemia resulting from either decreased production or increased loss. Twenty-five grams of albumin are roughly equivalent to 500 ml. of plasma. Salt-poor preparations are also commercially available. Plasma protein fraction U.S.P., which contains 90 per cent albumin and some gamma globulin, is also useful in the treatment of hypovolemia. Fluids should be concurrently administered with either preparation if the recipient is dehydrated. Neither requires cross-matching and both are relatively expensive. They may be stored at room temperature although less deterioration results at refrigerator temperatures. Both are heat treated to inactivate hepatitis virus.

Immunoglobulin replacement and therapy. Immunoglobulins are another product available from Cohn fractionation of plasma. They contain mostly IgG with little IgA or IgM. Immunoglobulin preparations have been used to prevent or modify hepatitis, rubella, and varicella infections. They have also been used for prophylaxis in patients with immunoglobulin deficiencies, in certain patients with malignancy, and with great success in the prevention of erythroblastosis fetalis.

Generally, only a small amount of any specific antibody is present in the standard gamma globulin preparations made from large pools. This may explain the lack of effectiveness of some preparations in preventing or modifying certain diseases. However, the preparations may contain appreciable titers of anti-A, anti-B, and anti-Rh_0.[136] The half-life of IgG is about 23 days and about one half is distributed extravascularly. Immunoglobulin preparations should be administered intramuscularly as serious reactions have occurred with intravenous administration. Treatment of immunoglobulin preparations with proteolytic enzymes has been shown to usually allow intravenous administration with little or no reaction[137]; most preparations available in the United States have not been treated in this manner.

Immunoglobulin prepared from plasma harvested from persons with high titers of specific antibody either following disease or deliberate immunization contains considerably greater amounts of the specific antibody desired and has been used to prevent varicella infections[138] and erythroblastosis fetalis.[140] A dose of anti Rh_0 (D) immunoglobulin of 20 μg per milliliter of red blood cells will effectively prevent Rh immunization[141] and the standard 300 μg dose of the substance given to nonimmunized, Rh-negative mothers delivering Rh-positive children is effective in preventing maternal alloimmunization in most instances. The number of fetal cells present in the maternal circulation following delivery may be estimated by the Kleihauer-Beke technique,[142] and if greater than usual numbers of fetal cells are present, more than the standard dose of the immunoglobulin should be administered.[141,143] The preparation should also be given to Rh-negative women following abortion and may be used to prevent alloimmunization following inadvertent transfusion of Rh-positive blood to an Rh-negative recipient.[143]

Immunoglobulins and prevention of hepatitis. Several studies with conflicting results have been performed by administering immunoglobulin preparations before and/or after transfusion or by mixing immunoglobulin with blood prior to transfusion in an effort to prevent post-transfusion hepatitis.[144-149] Prince and associates[150] have shown that most standard preparations of gamma globulin contain little antibody specific for the hepatitis B antigen, but that considerable variation occurs in the amounts of antibody present among various commercial preparations. Such differences of antibody content may explain divergent

results seen in clinical trials testing the effectiveness of gamma globulin in the prevention of hepatitis. A possible solution to the problem may involve the use of specific hepatitis B immune globulin which contains high-titer antibody directed against the hepatitis B antigen. Two large-scale clinical trials using this substance are currently under way in the United States,[151, 152] and there is possibility that use of this product may significantly decrease incidence of hepatitis after transfusion.

DISEASE TRANSMISSION BY BLOOD PRODUCTS

No discussion of component therapy would be complete without mention of diseases transmitted by transfusion. Although syphilis, malaria, brucellosis, and hepatitis have long been recognized as transmissible by administration of blood, only recently has there been appreciation of other agents such as cytomegalovirus and Ebstein-Barr virus[154, 155] as causes of morbidity and mortality. However, hepatitis reigns supreme as the major cause of transfusion-associated disease. It has been estimated that more than 30,000 cases of overt hepatitis resulting in 1,500 to 3,000 deaths occur yearly in the United States; The number of subclinical cases may be fivefold greater.[156] Although not all result from transfusion, both viral hepatitis type A (infectious hepatitis, MS-1, short-incubation hepatitis, epidemic hepatitis) and viral hepatitis type B (serum hepatitis, MS-2, long-incubation hepatitis, syringe hepatitis, "post-transfusion hepatitis") can be transmitted by blood transfusion. It has been estimated that from one third to as much as one half of hepatitis following transfusion may be due to hepatitis A virus.[157] The disease produced by hepatitis A virus tends to be less severe than that resulting from infection with hepatitis B virus, and deaths or subsequent severe liver disease are infrequent. Unfortunately, there is no laboratory test available to detect the presence of hepatitis A virus in blood products. Hepatitis B virus, on the other hand, may be strongly suspected when tests for hepatitis B antigen (hepatitis-associated antigen, Australia antigen), are positive. Some investigators feel that the hepatitis B antigen represents unused portions of viral lipoprotein coat synthesized by cells infected by the virus and not the virus itself.[158] Three types of virus-like particles have been observed by electron microscopy in blood containing hepatitis B antigen. The majority of particles are roughly spherical with a diameter of from 16 to 25 nm., although tubular forms with similar diameter but several hundred nanometers in length are also seen[159, 160]; it is thought that these represent that which is detected when blood is tested for the presence of hepatitis B antigen. A third type of particle, described by Dane and associates,[160] is larger and occurs relatively rarely; some have speculated that this particle may represent the causative agent of disease.

LeBouvier[161] has demonstrated the presence of three surface antigenic determinants on hepatitis B antigen. It appears that there are two mutually exclusive subdeterminants designated "d" and "y" in addition to a third designated "a" which is present on all particles; it has been postulated that types "ad" and "ay" represent antigenic determinants of two different types of hepatitis B virus. Other additional specificities have also been reported and eventually may be useful in epidemiologic studies. It is interesting to note that while the presence of hepatitis B antigen in donor blood varies widely throughout the United States (highest in the southeast and lowest in the north-central region), blood obtained from commercial sources more frequently transmits hepatitis than blood from volunteer donors.[162]

Detection of hepatitis B antigen and antibody. Although several different tests are being used for detection of hepatitis B antigen, they vary greatly in their ability to detect antigen and antibody (anti-hepatitis B antigen). There is no method known at present to detect blood containing hepatitis A virus. Of the currently employed tests for hepatitis B virus antigen and antibody detection, the ones most widely used are least likely to detect evidence of viral presence or exposure. Two-dimensional immunodiffusion is insensitive and takes 24 to 48 hours to perform but does allow both antigen and antibody presence to be confirmed by reactions of identity (Ouchterlony technique). At the present time, counterimmunoelectrophoresis is probably the most widely used screening test, although radiommunoassay techniques are expected to become the primary method in the very near future. The advantages of counterimmunoelectrophoreis over immunodiffusion include increased ease and speed of performance and improved antibody detection; it is probably severalfold more sensitive than immunodiffusion. Complement fixation, while even more sensitive for antigen detection, does not detect antibody as well, usually requires 48 hours to perform, and requires considerable technical skill. Hemagglutination or hemagglutination inhibition assays are even better detection systems, especially with respect to antibody, and are relatively easy to perform but are subject to problems associated with the carrier cell and with occasional false positive results due to the presence of certain serum immunoglobulins. Most workers agree that radioim-

munoassay using I^{125}-tagged reagents is the most sensitive test currently available; a detection kit is commercially available (Ausria, Abbott Laboratories). Solid phase radioimmunoassay may shorten testing time but is not without its own special problems. For a more comprehensive review of hepatitis, methods of detection, and methods envisioned to prevent the disease through immunization, readers may consult Vyas and associates.[163]

Even blood screened by the most sensitive techniques currently in use is capable of transmitting hepatitis, suggesting that even better tests must be devised or immunization procedures developed[164] if transfusion hepatitis is to be eliminated. It should be noted that the most widely used method of screening donor blood, counterimmunoelectrophoresis, is estimated to detect only one third of the bloods capable of tramsmitting the disease.[165] The risk of contracting hepatitis from transfusion is not clearly known; estimates vary widely based upon regional differences in viral incidence, methods of blood screening, the proportion of commercial and volunteer donors used, the proportion of previously transfused people who donate (who are nearly as likely to transmit disease as addicts[157]), the number of units transfused, and the basis upon which the diagnosis of hepatitis is made.

Hepatitis in blood components. All blood components, with the exception of gamma globulin, albumin, and plasma fraction U.S.P., are capable of transmitting hepatitis. Although heat treatment renders albumin and plasma protein fraction U.S.P. noninfective, a recent study suggests that hepatitis B antigen may be present in amounts necessary to initiate or provide a booster antibody response to the antigen.[166] Greatly increased risk is associated with the use of components prepared from pooled plasma, such as fibrinogen and factor IX concentrate. The use of single donor components, i.e., whole plasma as a source of factor IX and fresh-frozen plasma or cryoprecipitate as factor VIII sources, may decrease transmission of hepatitis in comparison with components prepared from large pools. Cryoprecipitate[167] is also useful for fibrinogen administration at less risk than commercially available preparations. Although concentrated components may very well be lifesaving in certain circumstances, they may not be needed except in those with a severe bleeding diathesis or at times of environmental or surgical trauma. Until such time as more sensitive tests become available or methods of active[164] or passive[153] immunization are proved, the transmission of hepatitis will remain a major complication of blood transfusion.

Phthalates. Phthalic acid esters are widely used in the manufacture of plastics to impart characteristics of workability and flexibility. Recently it has been demonstrated that certain of these esters migrate into blood or blood products during storage in polyvinyl chloride bags. Jaeger and Rubin[168] have shown that the amount of diethylhexyl phthalate present in blood stored 21 days ranged from 5 to 8 mg. per 100 ml.; the rate of accumulation appeared directly related to the time of storage. Human platelet concentrates contain even higher concentrations of the ester. Most of the material is associated with the lipoprotein fraction, although even washed red cell preparations contain measurable amounts of the substance.

The ester has also been found in tissue from autopsied patients who were transfused or had undergone cardiopulmonary bypass. The ability of the body to metabolize the ester to phthalic acid appears to vary among different people; thus quantities of the substance may accumulate in the frequently transfused patient. The long-term possible harmful effects of this compound are unknown, although work with animals has suggested it may be teratogenic.[169] Little is known of the possible harmful long-term effects of the accumulated ester in human beings; however, manufacturers of blood collection bags are currently attempting to develop compounds which do not impart this thoretically undesirable substance to collected blood.

REFERENCES

115. Triantaphyllopoulos, D. C.: Anticoagulant effect of incubated fibrinogen, Can. J. Biochem. 36: 2491, 1958.
116. Rymer, M. R., and Alsever, J. B.: Isohemagglutinins in pooled plasma, J. A. M. A. 202: 1109, 1967.
117. Kendrick, D. B.: Blood program during World War II, Washington, D. C., 1964, Office of the Surgeon General, Department of the Army, p. 781.
118. Cohn, E. J., Strong, L. E., Hughes, W. L., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L.: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids, J. Am. Chem. Soc. 68: 459, 1946.
119. Pool, J. G., Hershgold, E. J., and Pappenhagen, A. R.: High-potency antihemophilic factor concentrate prepared from cryoglobulin precipitate, Nature 203: 312, 1964.
120. Richerson, H. B., and Seebohm, P. M.: Anaphylactoid reaction to human gamma globulin, Arch. Intern. Med. 117: 568, 1966.
121. Henney, C. S., and Ellis, E. F.: Antibody production to aggregated human γ-globulin in acquired hypogammaglobulinemia, N. Engl. J. Med. 278: 1144, 1968.
122. Schorr, J. B., Ballard, S., and Radel, E: Hemolysis associated with "fibro-AHF" therapy of hemophilia. A discus-

sion of the mechanism and its possible prophylaxis, Transfusion 6: 522, 1966.
123. Johnson, A. J., Karpatkin, M. H., and Newman, J.: Clinical investigation of intermediate- and high-purity factor VIII concentrates, Br. J. Haematol. 21: 21, 1971.
124. Brinkhous, K. M., Shanbrom, E., Roberts, H. R., Webster, W. P., Fekete, L., and Wagner, R. H.: A new high-potency glycine-precipitated antihemophilic factor (AHF) concentrate; treatment of classical hemophilia and hemophilia with inhibitors, J. A. M. A. 205: 613, 1968.
125. Johnson, A. J., Aronson, D. L., and Williams, W. J.: Clinical use of plasma and plasma fractions, *in* Williams, W. J., Beutler, E., Erslev, A. J., and Rundles, R. W., editors: Hematology, New York, 1972, McGraw-Hill Book Company, Inc., pp. 1329-1353.
126. Biggs, R., and Bidwell, E.: A method for the study of antihaemophilic globulin inhibitors with reference to six cases, Br. J. Haematol. 5: 379, 1959.
127. Hirschman, R. J., Itscoitz, S. B., and Shulman, N. R.: Prophylactic treatment of factor VIII deficiency, Blood 35: 189, 1970.
128. Kasper, C. K., Dietrich, S. L., and Rapaport, S. I.: Hemophilia prophylaxis with factor VIII concentrate, Arch. Intern. Med. 125: 1004, 1970.
129. Hoag, M. S., Johnson, F. F., Robinson, J. A., and Aggeler, P. M.: Treatment of hemophilia B with a new clotting factor concentrate, N. Engl. J. Med. 280: 581, 1969.
130. Walsh, P. N., Rizza, C. R., Matthews, J. M., Eipe, J., Kernoff, P. B. A., Coles, M. D., Bloom, A. L., Kaufman, B. M., Beck, P., Hanan, C. M., and Biggs, R.: Epsilon-aminocaproic acid therapy for dental extractions in haemophilia and Christmas disease: A double blind controlled trial, Br. J. Haematol. 20: 463, 1971.
131. Storti, E., Ascari, E., Molinari, E., and Gamba, G.: Saving of cryoprecipitate in the surgery of hemophilic patients, N. Engl. J. Med. 287: 198, 1972.
132. Faria, R., and Fiumara, N. J.: Hepatitis B associated with Konyne, N. Engl. J. Med. 287: 358, 1972.
133. Weinreb, N. J., and Minsky, N.: Serious reaction after Konyne, N. Engl. J. Med. 285: 1151, 1971.
134. Edell, S.: Anaphylaxis after Konyne, N. Engl. J. Med. 285: 580, 1971.
135. Johnson, A., and Merskey, C.: Diagnosis of diffuse intravascular clotting: Its relation to secondary fibrinolysis and treatment with heparin, Thromb. Diath. Haemorrh. (Suppl.) 20: 161, 1966.
136. Niosi, P., Lundberg, J., McCullough, J., Park, B. H., and Biggar, D. W.: Blood group antibodies in human immune serum globulin, N. Engl. J. Med. 285: 1435, 1971.
137. Barundun, S., Kistler, P., Jennet, F., and Isliker, H.: Intravenous administration of human γ-globulin, Vox Sang. 7: 157, 1962.
138. Brunell, P. A., Ross, A., Miller, L. H., and Kuo, B.: Prevention of varicella by zoster immune globulin, N. Engl. J. Med. 280: 1191, 1969.
139. Pollack, W., Gorman, J. G., Freda, V. J., Ascari, W. Q., Allen, A. E., and Baker, W. J.: Results of clinical trials of Rh_0 GAM in women, Transfusion 8: 151, 1968.
140. Clarke, C. A.: Prevention of Rhesus iso-immunization, Semin. Hematol 6: 201, 1969.
141. Pollack, W., Ascari, W. Q., Crispen, J. F., O'Connor, R. R., and Ho, T. Y.: Studies on Rh prophylaxis. II. Rh immune prophylaxis after transfusion with Rh-positive blood, Transfusion 11: 340, 1971.
142. Mollison, P. L.: Blood transfusion in clinical medicine, Oxford, 1972, Blackwell Scientific Publications, pp. 696-697.
143. Pollack, W., Ascari, W. Q., Kochesky, R. J., O'Connor, R. R., Ho, T. Y., and Tripodi, D.: Studies on Rh prophylaxis. I. Relationship between doses of anti-Rh and size of antigenic stimulus, Transfusion 11: 333. 1971.
144. Grossman, E. B., Stewart, S. G., and Stokes, J., Jr.: Post-transfusion hepatitis in battle casualties and study of its prophylaxis by means of human serum globulin, J. A. M. A. 129: 991, 1945.
145. Mirick, G. S., Ward, R., and McCollum, R. W.: Modification of post transfusion hepatitis by gamma globulin, N. Engl. J. Med. 273: 59, 1965.
146. Drake, M. E., Barondess, J. A., and Bashe, W. J., Jr.: Failure of convalescent gamma globulin to protect against homologous serum hepatitis, J. A. M. A. 152: 690, 1953.
147. Holland, P. V., Rubinson, R. M., Morrow, A. G., and Schmidt, P. J.: γ-Globulin in the prophylaxis of post-transfusion hepatitis, J. A. M. A. 196: 471, 1966.
148. Prevention of posttransfusion hepatitis by γ-globulin: Preliminary report: A cooperative study, J. A. M. A. 214: 140, 1970.
149. Katz, R., Rodriquez, J., and Ward, R.: Posttransfusion hepatitis—effect of modified gamma globulin added to blood in vitro, N. Engl. J. Med. 285, 925, 1971.
150. Prince, A. M., Szmuness, W., Woods, K. R., and Grandy, G. F.: Antibody against serum hepatitis antigen: Prevalence and potential use as immune serum globulin in prevention of serum hepatitis infections, N. Engl. J. Med. 285: 933, 1971.
151. Roche, J. K., and Stengle, J. M.: Clinical trials of hepatitis immune globulin, N. Engl. J. Med. 287: 251, 1972.
152. Zimmerman, H. J.: VA study with hepatitis B immune globulin, N. Engl. J. Med. 287: 670, 1972.
153. Krugman, S., Giles, J. P., and Hammond, J.: Viral hepatitis, type B (MS-2 strain)—prevention with specific hepatitis B immune serum globulin, J. A. M. A. 218: 1665, 1971.
154. Prince, A. M., Szmuness, W., Millian, S. J., and David, D. S.: A serologic study of cytomegalovirus infections associated with blood transfusions, N. Engl. J. Med. 284: 1125, 1971.
155. Turner, A. R., MacDonald, R. N., and Cooper, B. A.: Transmission of infectious mononucleosis by transfusion of pre-illness plasma, Ann. Intern. Med. 77: 751, 1972.
156. Krugman, S.: Viral hepatitis and Australia antigen, J. PEDIATR. 78: 887, 1971.
157. Chalmers, T. C.: Carrier blood donors, *in* Vyas, G. N., Perkins, H. A., and Schmid, R., editors: Hepatitis and blood transfusion, New York, 1972, Grune & Stratton, Inc., pp. 277-288.
158. Purcell, R. H.: HBAg in blood donors, *in* Vyas, G. N., Perkins, H. A., and Schmid, R., editors: Hepatitis and

blood transfusion, New York, 1972, Grune & Stratton, Inc., pp. 312-313.

159. Hall, W. T.: Ultrastructural features of Australia antigen, *in* Vyas, G. N., Perkins, H. A., and Schmid, R., editors: Hepatitis and blood transfusion, New York, 1972, Grune & Stratton, Inc., pp. 226-233.
160. Dane, D. S., Cameron, C. H., and Briggs, M.: Virus-like particles in serum of patients with Australia-antigen-associated hepatitis, Lancet **1**: 695, 1970.
161. LeBouvier, G. L.: The heterogeneity of Australia antigen, J. Infect. Dis. **123**: 671, 1971.
162. Walsh, J. H., Purcell, R. H., Morrow, A. G., Chanock, R. M., and Schmidt, P. J.: Post-transfusion hepatitis after open heart operations: Incidence after the administration of blood from commercial and volunteer donor populations, J. A. M. A. **211**: 261, 1970.
163. Vyas, G. N., Perkins, H. A., and Schmid, R., editors: Hepatitis and blood transfusion, New York, 1972, Grune & Stratton, Inc.
164. Krugman, S., Giles, J. P., and Hammond, J.: Viral hepatitis, type B (MS-2 strain): Studies on active immunization, J. A. M. A. **217**: 41, 1971.
165. Kliman, A., Reid, N. R., Lilly, C., and Morrison, J.: Hepatitis-associated antigen (Australia antigen) in Massachusetts blood donors, N. Engl. J. Med. **285**: 783, 1971.
166. Redeker, A. G., Carpio, N. M., and Gocke, D. J.: Increased anti-HBAg titers after administration of human protein in solutions, N. Engl. J. Med. **287**: 102, 1972.
167. Hattersley, P. G., and Dimick, M. L.: Cryoprecipitates in treatment of congenital fibrinogen deficiency, Transfusion **9**: 261, 1969.
168. Jaeger, R. J., and Rubin, R. J.: Migration of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues, N. Engl. J. Med. **287**: 1114, 1972.
169. Marx, J. L.: Phthalic acid esters; biological impact uncertain, Science **178**: 46, 1972.