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THE Rh PROBLEM*
A Personalized History
(An example of the tsunami phenomenon in medicine)

Louis K. Diamond MD
Professor of Pediatrics, Emeritus, Harvard University
Adjunct Professor of Pediatrics
University of California, San Francisco

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Introduction

Years ago, when I was a junior physician at the Boston Children's Hospital, there came on our Service for further training a mysterious, quiet young man from Siam. He turned out to be of the royal family, and eventually became King Ananda of Thailand. Sometime later, another foreign house-officer appeared, also quiet, hard-working and able. We surmised from his imposing bearing that he too was royalty. We were not far wrong, for Dr. Doğramacı was even then a prince among all European pediatricians and their leader in his own country. It is a great privilege for me to give this lecture in his honor. I am happy to be here with him and his many students, friends and admirers.

Seldom in medical history has it been possible to present the complete story of a disease, from discovery through development of satisfactory treatment and finally even prevention, all accomplished within one generation. Since you are all familiar with the scientific facts, I plan to present mostly the history and some personal experiences with the Rh problem which has constituted a large portion of my medical activities for many years.

For me, the story began to unfold in 1932 when, on studying a group of stillborn and newborn infants with edema, anemia, and jaundice and reviewing the literature describing such cases under a variety of names, we realized these were all manifestations of a single disease process, called by pathologists erythroblastosis fetalis (EF).

Our review of 1932[1] lists more than 100 such cases reported from many parts of the world previous to that date when we published our own data on 20 infants.

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* Professor Louis K. Diamond was invited to give the first İhsan Doğramacı Lecture on April 9, 1979. His lecture inaugurated the series established by Hacettepe University, Ankara, Turkey, in honor of Professor İhsan Doğramacı, founder of that university and its rector from 1967 to 1975.

We did not uncover the etiology or pathogenesis although we should have. Opportunity knocked but our ears were not properly attuned. Thereby hangs a tale and from it, also a lesson.

In 1936, I cared for a newborn infant with anemia and jaundice and gave him a small transfusion of his father's blood. Although this was compatible by the standard cross match using red cells suspended in saline, it was rapidly destroyed in the child's circulation. With recurring anemia, the baby needed three more transfusions in the following week, all provided by his father, each helping him only briefly but also making him more jaundiced. When a fifth blood transfusion was desirable, his mother offered to give the needed 3 ounces. Her group 0 blood cross matched in the usual way, seemed compatible. To our consternation, the infant rapidly became more anemic and much more jaundiced. Review of the cross matching, using the standard 2% suspension of red cells in saline, showed no agglutination in the test tube of maternal serum vs. the infant's cells, but a hastily-prepared thicker red cell suspension, examined on a slide, seemed to have agglutinates. The tests were repeated by a consultant, a recognized expert in blood grouping. He too used 1-2% saline-diluted red cells and found no suspicious clumps. When shown my questionable agglutinates in a thicker smear, he criticized this as sloppy technique. He could not explain the infant's obvious transfusion reaction except to recall he had seen one before from the use of blood from a post partum donor and he said, "Women, especially during pregnancy, do queer things". Incidentally, he was a confirmed bachelor.

Had we pursued the questionable agglutinates in the cross match of the baby's red cells in the mother's serum, we might have discovered the maternal antibody as the cause of the infant's disease. *"Sic transit gloria mundi"*. The lesson from this might be: Experts should be consulted but their advice need not always be heeded.

As you know, seven years later in 1939[2] Philip Levine studied the blood of a woman who had recently delivered an infant with severe erythroblastosis fetalis and following transfusion from a compatible donor, her husband, she had a hemolytic reaction. In retrospect, it was fortunate her serum contained a rare saline-active antibody as well as the more common saline-inactive IgG antibody which is the cause of the disease. Levine, with keen insight, realized that here was a new blood group antigen, inherited by the infant from his father but absent in the mother. He reasoned she had formed antibodies to it and these, crossing the placenta, had caused severe hemolytic anemia or erythroblastosis fetalis in her baby. When in 1941 a somewhat comparable antibody was found by Landsteiner and Wiener[3] in rabbits and guinea pigs injected with Rhesus monkey red cells, the name Rhesus blood group (Rh for short) was born. Thus, from careful laboratory investigation by Levine of an unexplained transfusion reaction in a patient and by routine basic research in animals by Landsteiner and Wiener, a new field of study was opened up.

This one Rh antigen on red cells eventually flowered into 35 or more Rh variants and the 3 blood group families (ABO, MN and P) known in 1941 expanded into at least 10 major independent groups and dozens of minor uncommon ones. In all, there are now more than 400 red cell antigens known. Thus, work at the laboratory bench may yield results of inestimable clinical value.

One of the earliest problems we faced was: what induces Rh sensitization in an Rh negative woman? If about 15% of Caucasians are Rh negative and 85% are Rh positive, the incidence of Rh incompatibility between couples is about 13% and if this always induced maternal Rh antibodies, one of each 8 births could be an infant with EF. Actually, the statistics yielded an incidence of about 1 in 150, one-twentieth as many. Eventually it was found that the factors involved were numerous as shown in Table I.

TABLE I

Sensitization to Rh

1. Transfusion of Rh Positive Blood — 80% to 90% Effective
2. Pregnancy: Less than 1% to More than 11%

Important Factors:

A. Maternal vs. Fetal ABO Incompatible
(e.g.: mother, Group 0 vs. child, Group A)

B. Father, Rr (hetero-) or RR (homozyg.)
also, # Rh antigenic sites on rbc

C. Number of Rh Positive Pregnancies

D. Traumatic Delivery (including abortion)

E. Fetal-Maternal Transfusion
(1) Slow Leakage
(2) Large Fetal Bleed.

Although it was easy to recognize EF in an Rh positive neonate of an Rh negative woman, there was no simple test, before 1945, to detect Rh antibodies in the serum of the sensitized person or in the afflicted infant. In 1945 we developed the confirmatory rapid slide test[4] and then the useful albumin test[5]. In the same year Robin Coombs[6] of England described a highly sensitive antiglobulin test. Thereafter diagnosis of sensitization was simple, rapid and accurate. As so often happens, this new methodology led to unforeseen important advances in the fields of blood grouping, of immunology and of clinical medicine.

Next I turn to some historical background of the successful treatment of Rh problems. Our first concern was the rescue of the infant with EF. In 1946 and thereabouts, there were, conservatively, 200,000 such infants born in the United States yearly (of 4 million births). The mortality, even with early treatment by small transfusions, was close to 50%. Since the trouble lay in the infant's circulation where coated, damaged red cells were undergoing destruction and the plasma contained maternal Rh antibody, replacement transfusion was a logical procedure, but how to do this in a delicate newborn was a problem. Such exchange of blood was not new. It was actually carried out in 1667 by the first transfusionists of humans, Denis of France and Lower of England. Also Hart[7], a Canadian pediatrician had tried exchange transfusion for familial icterus gravis in 1925 using the anterior fontanelle puncture into the sagittal sinus to withdraw blood and a peripheral vein to inject it. Because this seemed hazardous, we experimented with steel needles and with rubber catheters in the umbilical vein, but clotting invariably hampered any successful removal and replacement of the infant's blood. Fortunately, plastic catheters became available at this time and being a nonwettable surface which practically prevented blood coagulation, we were able to carry out blood replacement or exchange transfusions via the umbilical vein late in 1946[8]. In succeeding years this has become the common route for sampling blood of newborns and for administering intravenous therapy. In fact, nowadays in an intensive care nursery, a neonate with catheters in the veins and an oxygen hood over the head may resemble an astronaut in training for a trip to the moon. Exchange transfusion quickly improved the prognosis for live-born neonates with EF. From 50% mortality before 1946, single exchange transfusions reduced the figure to 25% by mid 1949. By 1951 with monitoring of the bilirubin levels and multiple exchange transfusions, the mortality was about 12.5% and soon reached less than 5%[9]. The chief hazard thereafter was hyperbilirubinemia and the dread sequel of kernicterus (Table II) or nuclear brain damage. For prevention of this, exchange transfusion at certain levels of indirect bilirubin under various circumstances was advised. Originally, our charts showed curves for mandatory exchange transfusions at levels of 20 mg percent. With further experience, it was recognized that complicating factors such as acidosis, hypoalbuminemia, anoxia, etc., increased the possibility of developing kernicterus even at levels of hyperbilirubinemia below 20 mg percent. These more hazardous conditions are more likely to occur in sick newborns, particularly in prematures and in infants small for gestational age. The curves have therefore been redrawn to suggest exchange transfusion be given at lower levels of serum bilirubin when the complicating factors are present (Figures 1,2). Additional methods of treating hyperbilirubinemia have been developed, but when rapid lowering of the bilirubin levels is necessary, exchange transfusion is the method of choice[10].

Although the prognosis of liveborn infants with EF was greatly improved by prompt and repeated exchange transfusion and the supplementary therapy already men-

TABLE II

Kernicterus

(Toxic Damage to Nerve Cells by Unconjugated Hyperbilirubinemia)

Important Factors

1. Dangerous Level of Free Bilirubin:

   Greater than 20 mg % in Full Term "Normal" Newborn

2. Complicating Abnormalities:

Hypoalbuminemia	Hypoglycemia
Acidosis	Hypothermia
Anoxia	

3. Influencing Conditions:

   Low Birthweight (Immaturity)

   Drugs, Steroids, Free Fatty Acids

   Sepsis

tioned, there was still the loss in utero or at birth of 25% of infants of highly sensitized mothers. Such infants were either stillborn or died shortly after delivery and the severity of their disease process could not be predicted merely by measuring and following the maternal anti-Rh level during pregnancy. In 1950, Bevis of England[11] reported that the composition of the liquor amnii was altered in hemolytic disease of the newborn and in 1952[12] he recommended amniocentesis for measuring the pigments in the fluid in order to predict the severity of the hemolytic process. Liley[13] of New Zealand extended the usefulness of this analysis as a means of managing the course of the pregnancy. With the rise in pigment level in the amniotic fluid, early delivery might be indicated if the infant was sufficiently mature for sustained extra-uterine life. In 1963, Liley[14] further advised the trial of intrauterine transfusion of the endangered fetus too immature to be delivered. He injected compatible red cells through a needle or a catheter in the infant's peritoneal cavity. The resulting temporary improvement of the hemolytic anemia permitted intrauterine growth to proceed until gestational age and size made premature delivery safer.

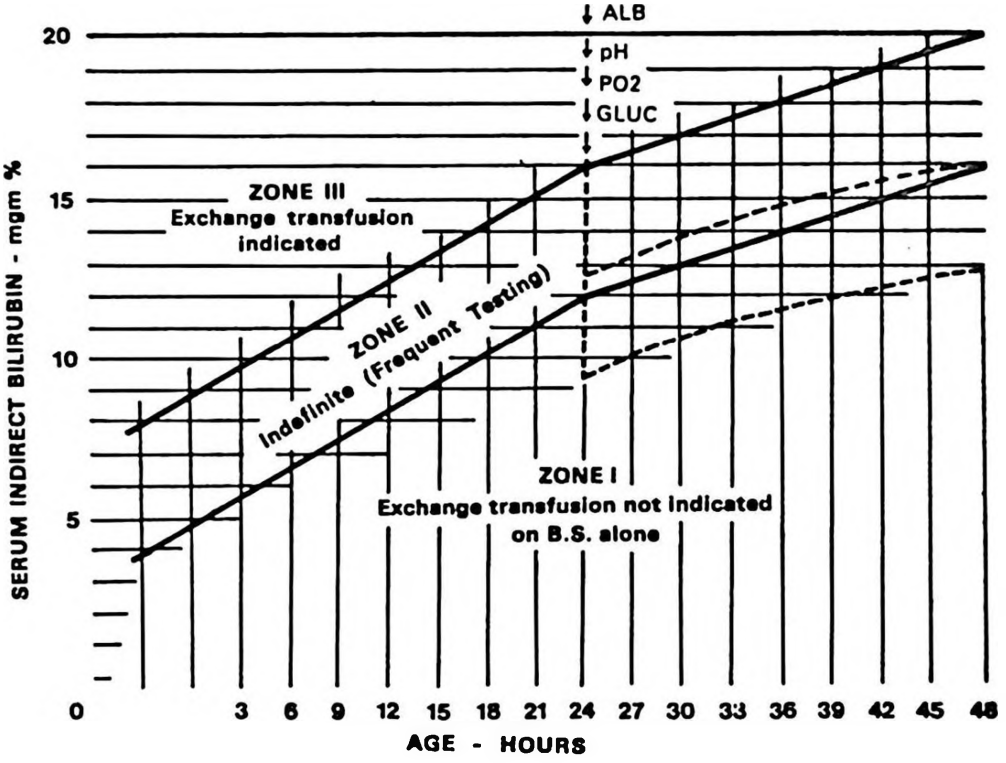

Figure 1

Mature Infants

Tentative Guide for Exchange Transfusion Based on Serum (Indirect) Bilirubin (B.S.)

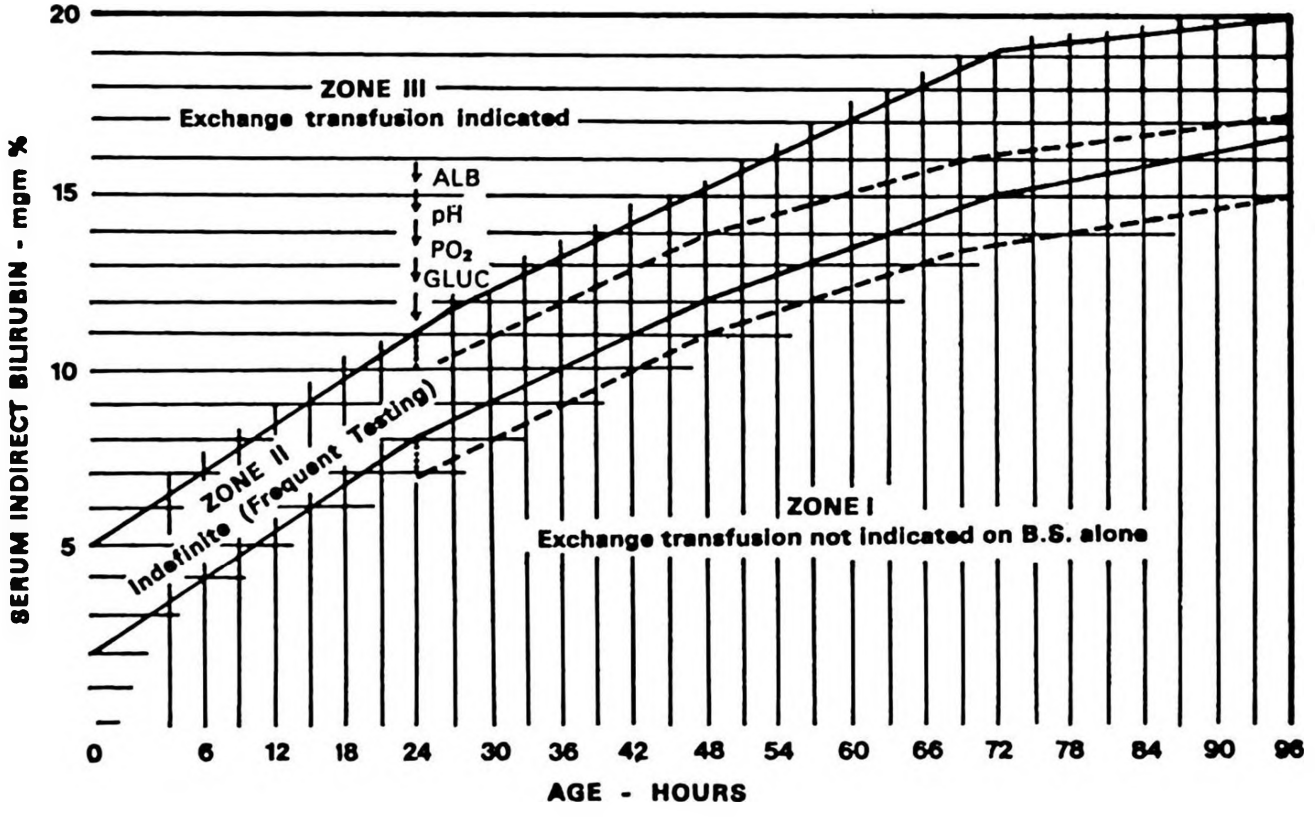

Figure 2

Premature Infants

Tentative Guide for Exchange Transfusion Based on Serum (Indirect) Bilirubin (B.S.)

Absorption of intact red cells from the intraperitoneal space, although it seemed a novel idea, was a time-honored approach to transfusion of anemic infants, used in the days before fine needles and thin catheters became available. In 1923 Siperstein[15] described transfusion by the intraperitoneal route in infants as did McKhann[16] in 1926. I actually performed such a transfusion, in 1927, in a very anemic one-year-old whose veins were collapsed; we raised his hemoglobin level from 30% to 60% in 24 hours.

Liley published simple graphs showing changes in amniotic fluid pigment measurements, with the indications for early delivery and for intrauterine fetal transfusion. Our own more elaborate and complicated chart (Figure 3) recommends precisely what is to be done at various gestational times and with different levels of pigment concentration in the amniotic fluid. As a result of these prenatal measures and the careful postnatal treatment of the afflicted infant, the incidence of the mortality in EF in utero has been reduced from over 25% to less than 10%, a remarkable improvement in cases that a dozen years ago were considered hopeless.

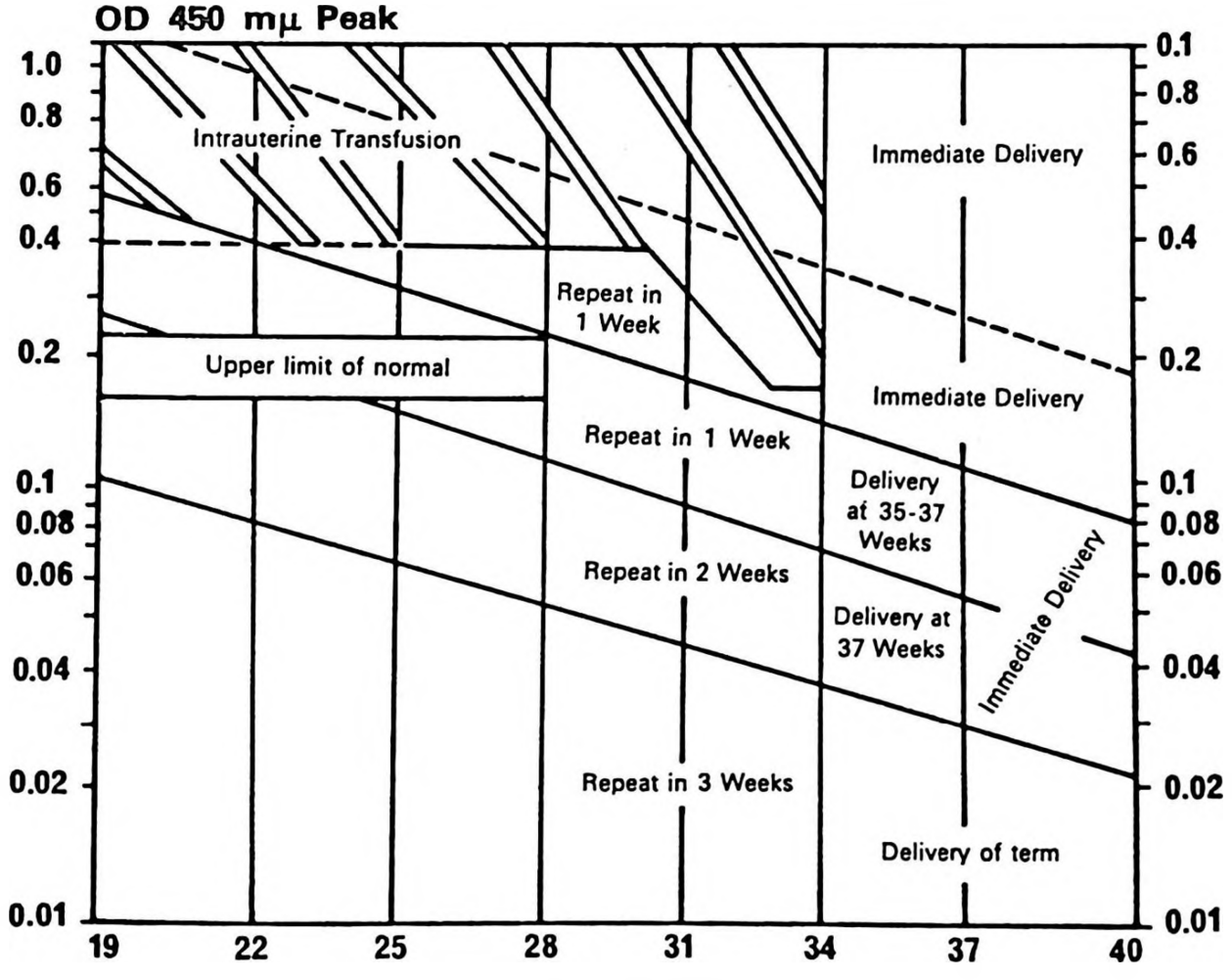

Figure 3

Amniotic Fluid Pigment Concentration

The final or more accurately the penultimate step in overcoming Rh incompatibility between mother and fetus and development of maternal antibodies causing EF, is only about 10 years old. In two centers, New York City and Liverpool, England, the successful prevention of maternal sensitization and antibody production against the Rh factor was developed at about the same time. It is interesting to consider briefly how this came about in each instance. In New York, Freda and his associates drew on the important principle established by Theobold Smith in the early 1890s. Smith showed that diphtheria toxin which ordinarily produced specific antibodies when injected into animals could be blocked from so doing by the simultaneous injection of antitoxin. Freda et al[17] tried administering anti-Rh serum together with or shortly after injection of Rh-positive red cells into Rh negative human volunteers. They proved that the same principle applied and sensitization to the Rh-positive cells, with production of antibodies against them, could be blocked completely[18]. Here again we see the value of perusal and knowledge of the literature and also the profitable cooperation among different specialists, such as the clinician, the immunologist, and the clinical pathologist. In England, Finn, Clarke et al[19] came on the same idea of blocking Rh-sensitization through recognition of what can again be called a successful experiment of nature. Levine[20] had pointed out many years before that Rh sensitization from an Rh positive fetus rarely occurred in an Rh negative woman of major blood group 0 when the fetus was group A. The logical explanation seemed that the maternal anti-A destroyed, or otherwise blocked, the group A Rh positive cells which happened to enter the maternal circulation either during pregnancy or more commonly at the time of delivery. Clarke and his group first tried intravenous injections of large amounts of anti-Rh serum and proved that the principle applied. Then, following the lead of Freda and Pollack, they used a gamma globulin concentrate of the anti-Rh serum which permitted as small a dose as one ml, intramuscularly, within two or three days after delivery of an Rh positive infant to an unsensitized Rh negative mother to protect her against sensitization and development of Rh antibody. This successful advance in preventive medicine, if properly applied in time and in sufficient dosage, should eliminate within a generation or two almost all of the pregnancy-associated Rh sensitization and make EF a vanishing disease.

In most cases, the number of fetal cells which enter the maternal circulation, chiefly at the time of delivery, is sufficiently low so that the usual dose of 0.5 ml of gamma globulin concentrate of anti-Rh serum given to the mother intramuscularly within 48 to 72 hours after delivery will protect against Rh sensitization. In the small number of cases where a larger "bleed" is suspected (birth of an anemic infant), an actual "count" of fetal cells (identified by the Kleihauer-Betke method of staining alkali- or acid-resistant Hb F) in the mother's blood smear will indicate whether a larger dose of the gamma globulin is necessary. Recently, Bowman et al[21] have initiated a program of treating unsensitized Rh negative women with the anti-Rh

gamma globulin concentrate at 28 weeks gestation as well as after delivery in order to counteract the leakage into the mother's circulation of a few Rh positive fetal cells. This treatment seems to be harmless to the baby as so little antibody enters the fetal system and may protect most of the Rh negative women who can become immunized during pregnancy (about 2% of all).

In summary, the steps in the conquest of EF (Table III) were first, the recognition of the disease through the use of a newly-discovered maternal antibody acting on the infant's and the father's red cells. Next, a series of laboratory tests were developed which confirmed the diagnosis and were important in prognosis of the disease and directing appropriate treatment. The third step, exchange transfusion, is the most rapid and effective measure, utilizing a plastic catheter in the umbilical vein.

TABLE III

Steps in Conquest of Erythroblastosis Fetalis

I. Recognition of Disease by New Test: Diagnosis through use of newly-discovered maternal antibody against infant's and father's red cells.

II. Simplification of Laboratory Test Methods:
   - A. Antibody test in saline solution
   - B. Antibody test in albumin solution
   - C. Slide test
   - D. Coombs' test
   - E. Microbilirubin test
   - F. Amniotic fluid measurement

III. Successful Treatment for EF:
   - A. Liveborn infant: 1. Transfusion - Rh negative blood
     2. Exchange transfusion
     3. Multiple Ex. Tx. (Prevention of kernicterus)
   - B. Potential stillbirth — intrauterine transfusion

V. Prevention — Use of Anti-Rh Gamma Globulin:
   - A. After birth of Rh-positive infant and after abortion
   - B. After incompatible transfusion

Repeated exchange transfusions may be necessary for the severely affected infant, particularly if hyperbilirubinemia develops and reaches dangerous levels with respect to toxic effects on the nerve cells (kernicterus). Additional measures for the control of milder degrees of hyperbilirubinemia are exposure to fluorescent lights and certain drugs like phenobarbital. For the support of infants in utero and prevention of potential stillbirths, intrauterine transfusion has generally been effective.

The fourth step, prevention of sensitization in the Rh negative unimmunized woman during and after gestation with an Rh positive infant, the use of a gamma globulin concentrate of anti-Rh serum has proven extraordinarily successful. It should be remembered that even a short-term pregnancy, ending in abortion or miscarriage, may initiate the formation of Rh antibodies. Treatment therefore has to be given in such cases in a manner similar to term pregnancy. Transfusion of Rh negative recipients with Rh positive blood either through laboratory errors or mistakes by personnel can be offset by prompt and adequate treatment with anti-Rh gamma globulin. The dose should be in sufficient quantity to "neutralize" the amount of red cells administered.

Ultimately, the conquest of EF and blood group antibody production awaits solution of the most difficult problem to date, that is, the neutralization of specific antibodies which have deleterious effects. Rh soluble substances or extracts to combine with specific Rh antibodies, but at the same time not stimulate further antibody production, have been suggested and occasionally tried. Unfortunately, the Rh antigen has not yet been obtained in pure form and in sufficient concentration and amount even for trial use. Research in this area is being pursued and the present generation of sensitized Rh negative women, still of childbearing age, may eventually be treated successfully with some such substance.

In conclusion, it is particularly interesting to review the advances that have been made in laboratory and clinical medicine as a result of the original finding of the Rh blood group and the basic research on new blood groups that resulted therefrom. Through the finding of the new Rh antibodies, it was possible first to identify the presence of the Rh antigens on human red cells. New testing methods uncovered still further new antibody production and eventually the finding of over a hundred new blood group antiserums. Thereby the several varieties of immunoglobulins were defined and eventually antibodies were identified, usually in multiparous women, against surface antigens of platelets and white cells; HLA typing, so important for the identification of individuals and successful transplantation from matched donors, developed from this.

The clinical applications of the basic research on blood groups and blood group antibodies are too numerous to describe. They are listed in Table IV.

If you have wondered about the strange word "tsunami" in the subtitle of this talk, I should explain that tsunami is the Japanese name for a huge tidal wave resulting

TABLE IV

Basic Research

On New Blood Groups

Rhesus RBC into Guinea PGS ⟶

Antibodies ⟶

Rh Factor on Human RBC

Clinical Research	Clinical Applications	Clinical Advances
New Testing Methods ⟶ New Antibodies Via: Albumin Medium Antiglobulin (Coombs') Enzyme Treatment	Etiology of EF and Kernicterus	Rescue of EF Fetus & Newborn Prevention of Hyperbilirubinemia
	Cause of Intragroup Transfusion Reactions	Millions of Safe Blood Txs
Discovery of > 100 New Blood Group Antiserums	Identification of Individuals via Profile of RBC Factors	Medicolegal, Anthropologic, and Genetic Characterization
Varieties of Immunoglobulins	Separation and Measurement of Immunoglobulin Fractions	Recognition and RX of Hyper-, Hypo-, A-Gammaglobulinemia
	Antibodies vs. all Blood Cells, Auto- and Allo-Immunoglobulins	Immune Hemolytic Anemia, Leucopenia, and/or Thrombopenia
Antibodies in Serums of Multiparous Women vs. Foreign Lymphocytes ⟶ HLA Typing	Tissue (HLA) Typing of Individuals (> 20 Types in 4 Major Systems)	Successful transplantation from Matched Donor

from an underwater earthquate or volcanic eruption, the importance of which is not appreciated when it originates, but it causes waves of tremendous size as it spreads to distant shores. Similarly, the discovery of the Rh factor created just a small ripple in the pool of research but eventually its ramifications were so important and widespread that it may be appropriately labeled "a tsunami phenomenon in medicine".

Finally, I have one more story. In 1680, there came to Cambridge, Massachusetts, from England a learned teacher and scientist, the Reverend Charles Morton. He was to be offered the Presidency of Harvard College which had had no president for 10 years. In 1970, the students had rebelled and forced the president's resignation. (You see how history repeats itself.) Understandably, Morton refused the position, but he taught all of the sciences at Harvard from a small book he had written, Compendium Physicae. This contained many aphorisms or terse brief sayings embodying a general truth. My favorite is: "All men had need of Physick(s) (meaning "medicine"), but Physitians should be herein not fidlers but Musitians". Dr. Doğramacı is indeed not an ordinary fiddler but a fine musician in medicine. Even more, he has proven he is a "Maestro" and can conduct large orchestras of medical practitioners and teachers whose number and talents are now beyond measure. For this, the country and the world are sincerely grateful.

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