

Section I

Developmental Hematology

Chapter

1

A Historical Review

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Ancient concepts of the blood were described by Hippocrates and Galen 2000 years ago in their doctrine of “humors.” It was believed that the body was made up of four humors – blood, phlegm, black bile, and yellow bile – and that these four components had the qualities of heat (hot-blooded!), cold, moist, and dry. The Galenic concept of the blood prevailed through the Middle Ages. Health or disease were a result of an imbalance, between these humors. This was the basis of the practice of therapeutic bloodletting (which, fortunately, was performed infrequently on children) through the mid nineteenth century as a way to rid the body of the imbalance of humors believed to cause a wide variety of diseases.

The hematology of the fetus and newborn is a relatively recent area of study whose development depended upon the evolution of the science of hematology and, especially, upon methods to study the blood and its elements. As Wintrobe has pointed out, the development of the field of hematology has been driven by technology. He divided the evolution of hematology into two general areas: *morphology*, which relied on the development of microscopy, and *quantitation* of the elements of the blood, which came later [1].

The invention of the microscope enabled identification of the blood cells. Antonie van Leeuwenhoek, working in Delft, Holland, constructed a primitive microscope from the minute biconcave lens mounted between two metal plates attached to a screw that permitted focusing. Leeuwenhoek’s publication in 1674 contained the first accurate description of the red blood corpuscles [2]:

The blood is composed of exceedingly small particles, named globules, which in most animals are

red in color . . . These particles are so minute that 100 of them placed side by side would not equal the diameter of a common grain of sand.

In the centuries following, the development of compound microscopes with two lenses greatly increased magnification and minimized spherical aberration, permitting more accurate descriptions of the blood cells. Dr. William Hewson, who has been designated as one of the “fathers of hematology,” noted that the red cells were flat rather than globular and also described the leukocytes for the first time [3]. The last of the formed elements of the blood, the platelet, was recognized independently by several investigators. The most definitive early work on the platelet was done by Giulio Bizzozero. His monograph, published in 1882, clearly recognized these cells as being distinct from red and white blood cells, and suggested that they should be called *Blutplättchen*. He also assigned a hemostatic function to the platelet [4]. Dr. William Osler, early in his illustrious career, also described platelets accurately, although he believed that they may be infectious agents, perhaps analogous to bacteria [5].

With improvements in microscopy, the morphology of the fixed blood cells began to be examined using thin films of blood, spread and dried on glass slides, which were then stained with aniline dyes that stained differentially the nuclei and granules of the leukocytes. Staining of peripheral blood smears was developed by Paul Ehrlich in 1877, while he was still a medical student [6], and became practical in the early twentieth century by the work of Dr. James Homer Wright of Boston, who formulated the polychromatic Wright stain that is still used today for morphologic examination of the blood and bone marrow. The

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development of supravital dyes provided a method for assessment of erythropoiesis by reticulocyte counts. These techniques permitted the flowering of morphologic hematology, and many blood diseases such as the leukemias and the various types of anemia were described on the basis of typical morphological findings.

Hematology as a quantitative discipline began with the development of practical and reliable methods to quantify accurately the numbers of the various blood cells. These methods used gridded chambers of uniform depth (hematocytometers) into which precisely diluted suspensions of blood were placed. The numbers of cells in the chamber were counted and, when combined with the known dilutions, the actual numbers of cells per cubic milliliter in the patient's blood could be calculated. Hemoglobin levels were estimated by comparing the density of color in fixed dilutions of hemolyzed blood with colorimetric standards and, later, by spectroscopy. For many years, hemoglobin values were reported as "% of normal"; and because the definition of "normal" was often different there was considerable variability from study to study. In 1929, Dr. Maxwell Wintrobe described his method for obtaining the hematocrit or packed red-cell volume (PCV) by centrifugation of blood in a glass tube [7]. He then defined so-called red-cell indices, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), which proved of enormous value in classifying the various forms of anemia [8]. The latest advance in blood-cell quantitation began in the 1950s with the introduction of increasingly more complicated and sophisticated computer-driven electronic instruments that measure hemoglobin very accurately, the numbers of all the blood cells, as well as the red-cell indices and the red-cell distribution width (RDW). Some instruments now also provide automated differential counts of the leukocytes.

Most of the pre-twentieth century American pediatric textbooks gave scant attention to hematologic problems of the neonate. Dr. W.P. Dewees's 1825 *A Treatise on the Physical and Medical Treatment of Children*, arguably the first American pediatric textbook, and Dr. Job Lewis Smith's 1869 *A Treatise on the Diseases of Infancy and Childhood* gave only passing notice to blood conditions of the neonate, such as neonatal jaundice and hemorrhage from improper ligation of the umbilical cord [9, 10]. However, the monumental pediatric text of

Dr. L. Emmett Holt, *The Diseases of Infancy and Childhood*, first published in 1897, contained a section on "the Diseases of the Newly-Born," including the hemorrhagic disease, and a 17-page section on "the Diseases of the Blood," which included the normal blood findings in the newborn [11]. Holt was obviously familiar with the many studies published in the German literature, and his descriptions are reasonably consistent with modern findings:

The percentage of hemoglobin is highest in the blood of the newly born . . . At this time the number of red blood corpuscles is from 4,350,000 to 6,500,000 in each cubic millimeter . . . In size, a much greater variation is seen in the red cells of the neonate. In the blood of the foetus there are present nucleated red corpuscles or erythroblasts. These diminish in number toward the end of pregnancy. These are always found in the blood of prematures, but in infants born at term, they are seen only in small numbers. The number of leukocytes in the blood of the newly born is three or four times that of the adult, being on the average 18,000 per cubic millimeter.

In 1921, Dr. W.P. Lucas and associates from the University of California Medical School in San Francisco described their extensive studies of the blood of 150 infants at birth and during the first 2 months of life [12]. Their samples were obtained from serial punctures of the longitudinal sinus! The polycythemia of the newborn and changes in the leukocytes were defined clearly.

In 1924, Dr. H.S. Lippman from the University of Minnesota published detailed studies of the blood of newborn infants [13]. He noted (without further details) that "Denis published the first observations on the subject in 1831." Lippman's review of the literature cited 70 previous articles on the hematology of the newborn. Most of these studies were published in European, especially German, journals. Although there was considerable variability because of different methods and standards, the consensus of these early studies was that "Hemoglobin values at birth are higher than at any other period in the children's life." Some of these studies described reticulocytosis and normoblastemia in the first day of life, which declined rapidly in the first week of life. Lippman conducted serial studies of capillary blood over the first 48 hours of life in 71 normal newborns as well as changes in the leukocytes during this period.

It has been known for 100 years that the red-blood cells (RBC) of the fetus and newborn are large compared with those of adults, as determined by microscopic measurement of red-cell diameter. Newer electronic cell-sizing techniques have demonstrated that the mean MCV of the neonate's red blood cells averages 110 fl, compared with the 90 fl of adults. The red cells of midgestational fetuses are even larger [14].

In 1856, E. Korber, in his doctoral dissertation, is reported to have described his experiments that showed that solutions of the hemoglobin of newborn infants resisted denaturation by strong alkaline solutions and maintained a red color, while hemoglobin solutions from adults treated in the same way were rapidly denatured and decolorized [15]. The property of alkali resistance became the basis of the Singer one-minute alkali denaturation test for quantitation of fetal hemoglobin (Hb F), as well as the Apt test, used to differentiate fetal from swallowed maternal blood in infants with gross blood in the gastrointestinal tract [16, 17]. Fetal hemoglobin is also resistant to acid denaturation, which is the basis for the red-cell acid elution staining procedure of Drs. C. Kleihauer, H. Braun, and K. Betke that is used widely to quantitate the magnitude of fetomaternal transfusions [18].

The understanding of the protein structure of hemoglobin advanced rapidly in the 1950s when it was shown that adult hemoglobin, Hb A ($\alpha_2\beta_2$), is a tetramer of alpha (α) and beta (β) polypeptide chains and that Hb F ($\alpha_2\gamma_2$) contains a different pair of polypeptide chains designated as gamma (γ) chains [19, 20]. During fetal development, synthesis of γ chains predominates, but with approaching term there is a fall-off of γ -chain synthesis and a simultaneous reciprocal increase in β -chain synthesis. The regulatory mechanisms that govern this " β/γ switch" remain to be elucidated. The blood of the newborn contains large amounts of Hb F, averaging 60%–80%. The affinity of Hb F for oxygen is greater than that of Hb A because of poor binding of 2,3-diphosphoglycerate. This results in a shift of the oxygen dissociation curve to the left, which is favorable for oxygen transport to the fetus in the relative hypoxia of intrauterine existence but which may be disadvantageous after birth [21]. The high level of Hb F at birth offers temporary protection from β hemoglobinopathies, such as sickle cell anemia, and may hamper their diagnosis in the newborn.

Roland Scott, using the relatively insensitive "sickle cell prep," demonstrated a much lower frequency of "sicklelemlia" in black newborns than was found in older children from the same community [22]. The development of techniques such as acid agar gel electrophoresis and high pressure liquid chromatography have permitted genotypic diagnosis of most hemoglobinopathies at birth, and neonatal testing for hemoglobinopathies is now performed routinely in all 50 states of the USA [23].

The only somewhat common hemoglobinopathy that produces symptoms in the newborn is homozygous alpha-thalassemia resulting from deletion of four alpha-globin genes [24]. In parts of Southeast Asia, fetal hydrops is caused much more frequently by alpha-thalassemia than by Rh immunization. The recent immigrations of large numbers of Southeast Asian people into the USA have resulted in increasing numbers of affected infants. Some of these have survived after intrauterine transfusions but are transfusion dependent [25].

Other inherited conditions such as hereditary spherocytosis, a defect in RBC spectrin, may be associated with non-immune hemolysis, both in utero and in the neonatal period, resulting in anemia and hyperbilirubinemia. Inherited deficiency of RBC enzymes such as glucose 6-phosphate dehydrogenase combined with exposure to oxidant substances, perhaps fava beans, causes epidemic neonatal hyperbilirubinemia in Greek infants.

Since the turn of the twentieth century, a large number of studies of the hematology and blood diseases of the newborn have been reported. Much of this information has been incorporated into textbooks of hematology. Dr. Maxwell Wintrobe's monumental *Clinical Hematology*, which was first published in 1943, contained sections on normal blood values, anemias, and hemorrhagic diseases of the newborn. Neonatal thrombocytopenia in infants born of mothers with immune thrombocytopenic purpura (ITP) was also mentioned briefly. In subsequent editions of Wintrobe's text, many more neonatal hematological conditions were described. In 1960, Dr. Carl Smith published *Blood Diseases of Infancy and Childhood*, the first American textbook of pediatric hematology/oncology. This had several chapters devoted to normal values and hematologic problems in the neonatal period.

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In 1966, Drs. Frank Oski and Laurie Naiman published *Hematological Problems in the Newborn*, the first text devoted solely to the hematology and hematological problems of the neonate [26]. The authors' stated purpose was

... to provide in a single source much of what is known concerning both the normal and abnormal hematologic processes of the first month of life and the effects of prenatal factors on them ... And to provide a useful guide to all who care for the newborn infant – those who are continually confronted with infants who are bleeding, anemic or jaundiced.

The Oski–Naiman text had two subsequent editions in 1972 and 1982. Subsequently, there have been a plethora of texts and handbooks on pediatric hematology, most of which devote chapters to the newborn.

The history of neonatal hematology and the process of understanding hematologic diseases based on clinical and laboratory observations that stimulate investigation of basic mechanisms and then therapeutic interventions are illustrated well by three quintessential neonatal blood conditions: *erythroblastosis fetalis*; *hemorrhagic disease of the newborn*; and *physiological anemia of infancy*.

Erythroblastosis Fetalis

As recently as 1946, erythroblastosis fetalis, or hemolytic disease of the newborn, affected between 0.5% and 1.0% of fetuses and newborns in the USA. It had a 50% mortality as well as significant neurologic morbidity in many survivors [27]. Prior to 1936, three seemingly distinct neonatal syndromes had been identified: *fetal hydrops*, *icterus gravis neonatorum*, and *anemia of the newborn*. Based on histological and hematological similarities, Drs. L.K. Diamond, K. Blackfan, and J. Baty advanced a unifying hypothesis that these three syndromes were manifestations of a single underlying disease process. They designated all of these neonatal syndromes “erythroblastosis fetalis” because of massive nucleated RBC proliferation in the organs and blood [28].

Dr. Ruth Darrow was a pathologist who had several of her own children die of erythroblastosis. In 1938, she advanced a brilliant inductive hypothesis about its cause. Assembling all of the available

information, as well as drawing on her tragic personal experiences, she noted the usual sparing of the first child and the progressive involvement of most subsequently born children. She recognized that the clinical, hematologic, and histopathologic findings in these infants could be best explained by severe hemolysis. She concluded that the disease results because [29]:

The mother is actively immunized against fetal red cells or some component of them ... The antibodies formed in the maternal organism may then pass to the child through the placenta.

The elusive offending red-cell antigen and its antibody were discovered in 1940 by Drs. Karl Landsteiner and Alexander Weiner. It was given the name Rh (rhesus factor) because the antibody was produced by injection of red-blood cells of rhesus monkeys into rabbits. This antibody agglutinated the red cells of 85% of normal individuals [30]. Interestingly, Landsteiner's discovery of the Rh blood group was accomplished almost 40 years after he had discovered the ABO blood groups [31]. In 1941, Dr. Philip Levine and associates described a severe transfusion reaction in a postpartum woman who received a transfusion of her husband's blood shortly after delivering a stillborn baby with hydrops fetalis. Levine was able to demonstrate Rh antibodies in the mother's circulation, defining clearly the pathophysiology of erythroblastosis fetalis [32, 33].

Effective treatment for erythroblastosis progressed slowly. The treatment of icterus gravis by “exsanguination transfusion” was first reported in 1925 by Dr. A.P. Hart at the Toronto Hospital for Sick Children [34]. With the discovery of the Rh factor, exchange transfusion evolved rapidly as a way to remove circulating antibodies, sensitized red-blood cells, and bilirubin; Drs. Harry Wallerstein and Alexander Weiner in New York and Louis K. Diamond in Boston spearheaded this treatment. Wallerstein's method involved aspiration of the neonate's blood from the sagittal sinus and infusion of Rh negative blood into a peripheral vein [35]. Weiner's method employed heparinization and surgical cannulation of the radial artery and saphenous vein. Interestingly, at a time long before institutional review boards for research, he first evaluated the technique in a nonerythroblastic “Mongolian idiot” [36]. Diamond's much more practical method utilized the umbilical vein to alternately remove and

infuse blood, and this rapidly became the accepted method around the world [37]. Drs. Diamond and F. Allen developed practical guidelines for the prenatal and postnatal management of Rh-sensitized mothers and their erythroblastotic newborns. These reduced neonatal mortality from 50% to 5% and intrauterine death from 20% to less than 10%.

Kernicterus associated with severe hyperbilirubinemia was virtually eliminated [38]. Implicit in the pathogenesis of Rh erythroblastosis is that small numbers of fetal erythrocytes gain entrance into the maternal circulation, particularly during labor, where they evoke maternal immunization and Rh antibody formation. The possibility of large fetomaternal transfusion was first hypothesized by Dr. A. Weiner and proven definitively by Dr. Bruce Chown, who used differential agglutination to demonstrate and quantitate fetal red cells in the maternal circulation in a case of neonatal anemia [39, 40]. It is now recognized that acute, massive fetomaternal transfusion during labor can result in severe anemia, neonatal pallor and hypovolemic shock resembling asphyxia pallida. Chronic fetal/maternal hemorrhage during pregnancy may be associated with a well-compensated congenital microcytic hypochromic anemia due to iron deficiency because of chronic blood loss by the fetus [41].

Two penultimate important developments in erythroblastosis fetalis were provided by Dr. A.W. Liley of New Zealand, who devised a method of spectroscopic analysis of amniotic fluid to determine increase in fetal bilirubin as a result of hemolysis. This enabled identification of immunized fetuses who were at high risk of intrauterine death. Liley showed that these severely affected infants could be given intrauterine intraperitoneal transfusion of Rh negative RBC to carry them to delivery [42, 43]. Development of percutaneous umbilical blood sampling under ultrasonographic guidance has enabled perinatologists to directly diagnose and assess the severity of anemia in immunized fetuses and to treat them with simple or exchange transfusion in utero.

In 1967, Drs. C.A. Clark in Liverpool and V.J. Freda and associates in New York showed independently that primary isoimmunization of Rh-negative mothers by the Rh-positive red cells of their fetuses could be largely prevented by immediate postnatal administration of potent anti-Rh gamma globulin to the mother [44, 45]. In most of the developed world, erythroblastosis

fetalis has become a rare disease of largely historical interest, and exchange transfusion has become a lost skill [46].

Hemorrhagic Disease of the Newborn

Newborn infants may bleed seriously from several causes. More than 2000 years ago, the familial occurrence of severe bleeding following ritual circumcision of boys, who doubtless had hemophilia, was recognized [47]:

It has been reported of four sisters at Sepphoris, the first one circumcised her son, and he died, the second, and he died; the third and he died. The fourth came before R. Simeon b. Ganaliel who said to her abstain from circumcision . . . for there are families whose blood is loose; while in others it coagulates.

(Babylonian Talmud, Tracate Yevamoth, fol. 64b)

Dr. Armand Quick, in a 1942 review of the history of coagulation, noted that possible cases of a neonatal hemorrhagic disease, distinct from hemophilia, had been reported as far back as 1682. Quick also postulated that the delay of ritual circumcision by Jews until the eighth day of life may have been based on their empirical observations that neonatal bleeding symptoms have largely waned by that time [48].

However, the first definitive description of “the haemorrhagic disease of the newborn” was provided by Dr. C.W. Townsend in Boston in 1894. Townsend described a generalized, not local, bleeding disorder beginning on the second or third day of life. About 0.6% of newborns were affected with clinical hemorrhage, chiefly into the skin, gastrointestinal tract, and central nervous system. There was a 62% mortality rate, but if not fatal, the disease was self-limited, with most cases recovering within 5 days. The sexes were affected equally [49]. The onset of transient bleeding only in the first few days of life, as well as the involvement of girls, clearly differentiated hemorrhagic disease of the newborn from hemophilia.

Dr. W.P. Lucas and associates performed serial clotting times, a measure of the entire coagulation mechanism, in newborns and showed that during the first four days of life “there is a definite and fairly consistent prolongation of the coagulation time which favors the so called

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hemorrhagic condition of the newborn” [12]. Dr. G.H. Whipple in 1912 showed that the plasma of infants with hemorrhagic disease was deficient in prothrombin [50], and this deficiency was corroborated and expanded by Drs. K.M. Brinkhaus and associates in 1937 [51].

Treatment of hemorrhagic disease of the newborn was essentially limited to supportive measures, including local compression when possible [11: p. 104]. More than half of affected babies died of intracranial hemorrhage or hemorrhagic shock. Dr. S.W. Lambert in 1908 was able to rapidly reverse the bleeding of an affected baby by a transfusion in which the father’s radial artery was anastomosed to the baby’s popliteal vein [52]. In 1923, Dr. J.B. Sidbury, a practicing pediatrician in North Carolina, successfully treated the hypovolemic shock as well as the bleeding disorder of an affected newborn by giving a blood transfusion through the umbilical vein. Sidbury stated that “human whole blood has acted as a specific in this condition” [53]. In the 1920s, and continuing into the 1940s, the standard treatment of hemorrhagic disease of the newborn, and in some centers the prophylaxis of the condition, was the intramuscular injection of adult blood, often obtained from the father. This was before the discovery of the Rh factor, and this led to the Rh immunization of some girls and subsequent erythroblastosis fetalis in their offspring [54].

Understanding of the pathogenesis of hemorrhagic disease of the newborn was made possible in 1929 when Dr. H. Dam and associates showed that chicks fed an ether-extracted diet developed a severe bleeding tendency that could be prevented by feeding material they extracted and purified from cereals or seeds. They named the correcting factor “Koagulations-vitamin,” or vitamin K [55, 56]. The nature of the bleeding defect in vitamin K-deficient chicks was soon localized to a deficiency of prothrombin and defined clearly by Brinkhaus and colleagues and Dam and colleagues in normal babies and those with hemorrhagic disease [57, 58]. Dr. W.W. Waddell and associates showed that vitamin K administration could prevent coagulation abnormalities in newborns [59]. Synthesis of vitamin K was accomplished in 1939 [60]. Routine vitamin K prophylaxis (0.5–1.0 mg given subcutaneously) for all newborns was recommended by the Committee on Nutrition of the American Academy of Pediatrics in 1961, and hemorrhagic disease of the newborn has virtually

disappeared in the developed world [61]. It should be mentioned, however, that the incidence of hemorrhagic disease of the newborn had decreased markedly in the USA even before vitamin K prophylaxis became routine. This decrease was probably a consequence of the declining incidence of breast feeding from the 1930s through the 1960s. The vitamin K content of breast milk is much lower than that of cows’ milk and hemorrhagic disease of the newborn occurs almost exclusively in breast-fed infants who, deliberately or inadvertently, do not receive prophylactic vitamin K [62, 63]. The biochemical basis of the action of vitamin K has been shown to relate to the gamma-carboxylation of glutamine acid residues in the vitamin K-dependent coagulation factors, including prothrombin [64].

Physiological Anemia of Infancy and the Early Anemia of Prematurity

At birth, the concentration of hemoglobin of full-term infants averages 16.4 gm/dL, higher than it will ever be during a lifetime. This relative polycythemia of the newborn is attributable to the low arterial PaO₂ in utero that stimulates erythropoietin (EPO) production with a consequent high rate of erythropoiesis. In the fetus and neonate, most EPO production occurs in the liver rather than in the kidney. After birth and the establishment of respiration, the arterial PaO₂ rises, triggering sharp declines both in EPO production and the rate of erythropoiesis. The dampened red-cell production is reflected in the disappearance of circulating nucleated RBCs and a fall in the reticulocyte count that continues for 6–8 weeks. During this period, the red-cell life span is only about 90 days, compared with 120 days in adults [65]. As a consequence of continuing RBC destruction and lack of production, hemoglobin levels in the term baby fall steadily from about 16.4 gm/dL to about 11.0 gm/DL at 6–8 weeks of age. This fall, which reflects the physiologic transition from the relatively hypoxic intrauterine environment to the oxygen replete extrauterine state, is appropriately called the *physiological anemia of infancy* [66].

After 6–8 weeks, red cell production resumes as indicated by a rise in reticulocyte count, resulting in stabilization of the hemoglobin level, and

then an increase in hemoglobin level that plateaus at an average of 12.5 gm/dL for the first 5–6 years of life. The rate of RBC production is sufficient to maintain a stable hemoglobin level during childhood despite the increase in blood volume that occurs in the first years of life because of the expanding blood volume that accompanies growth.

In the preterm infant, the postnatal fall in hemoglobin concentration is more marked and occurs sooner than in the full-term infant – the smallest infants having the greatest decline in hemoglobin concentration. This is called the *early anemia of prematurity*, which is in part an exaggerated physiological anemia. Premature infants have inappropriately low EPO levels for the severity of the anemia, perhaps because hepatic EPO production is less sensitive to reduced oxygen concentrations relative to the renal production mechanism [67]. It was thought that administration of EPO might be specific therapy that would modify the degree of anemia and reduce the need for RBC transfusion. However, the possible clinical benefit of EPO therapy is controversial and today the drug is not usually routinely administered to small premature infants [68]. Other factors that contribute to the early anemia of prematurity include a very large expansion of blood volume (so-called “bleeding into the circulation!”), a red-cell life span that is even shorter than in term infants, and especially the relatively large amounts of blood often removed for laboratory studies in sick, premature infants.

Epilog

This review of the history of neonatal hematology shows clearly that the study of the blood of the fetus and newborn has captured the attention of pediatricians and hematologists over many years. It is surprising how large their contributions were and that “there is nothing new under the sun.” The sagas of erythroblastosis fetalis and hemorrhagic disease of the newborn and the understanding of the physiological anemia of infancy illustrate the progress from clinical recognition and description, to definition of pathogenesis, to empiric and then specific therapy, and finally in many instances to prevention.

The majority of work and investigation in neonatal hematology has been performed by pediatric hematologists. However, there is now

an emerging generation of neonatologists who have been trained in clinical and investigational hematology, who work in newborn special care units, and who have made hematology their clinical and research focus.

As we examine neonatal hematology today, the morphological and quantitative studies of earlier eras of hematology have been succeeded by a modern era of genetic, biochemical, and molecular investigations of the processes that regulate the fetal and neonatal blood and may result in diseases when they go awry. Discoveries in these areas will revolutionize neonatal hematology and, hopefully, lead to ever more effective interventions in this vulnerable population.

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