RED CELL METABOLISM AND NEONATAL JAUNDICE *

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Jaundice is as old as medicine. It was one of the first definite signs of illness recognized by physicians of ancient times. Approximately 60 per cent of newborn infants show jaundice during the first few days of life. Most of the time it is mild, appearing clinically on the second or third day in full term newborns and somewhat later in premature infants. However, early investigators noticed that in a relatively small number of cases, jaundice would appear at birth, or soon after, and, following a few days of progressively increasing icterus, the infant would either die or, if he survived, be physically or mentally crippled for life. This latter from was called *icterus garvis,* while the milder from was called physiological jaundice of the newborn. This term, however, seems inappropriate now, in view of recent findings indicating that increased breakdown of neonatal erythrocytes is not the only cause of jaundice. A temporary defect in bilirubin metabolism specific for this period of life is the most important cause of jaundice in the newborn.

Whatever the cause may be, when total serum bilirubin levels exceed 12 mg per 100 ml during the first day of life, the condition is called neonatal hyperbilirubinemia. Although in some of the jaundiced newborns the elevated serum bilirubin may be mostly of the direct-reacting or conjugated variety (e.g. obstructive jaundice), the following discussion will deal mainly with neonatal jaundice due to the accumulation, in blood, of indirect-reacting bilirubin. Since it is known that hyperbilirubinemia of the indirect variety may result in kernicterus, many studies have been done to detect the possible factors causing high bilirubin levels in the newborn. In general, it may be said that hyperbilirubinemia is the result of either excessive breakdown of red cells which results in an increase of indirect-reacting bilirubin in the blood, or the failure to convert

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* This work was supported in part by a grant from Eczacıbaşı Drug Co., Istanbul.

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indirect-reacting bilirubin to the direct-reacting or water soluble variety. In some cases, both mechanisms may be involved. Most of the known factors acting through one or both of the mechanisms are listed in Tables 1 and 2. However, it must be emphasized that by far the most frequent causes of neonatal hyperbilirubinemia are the blood-group incompatibilities such as Rh and ABO. In the United States, for instance, the incidence of hyperbilirubinemia without blood-group incompatibilities is about five per cent among full term newborns and is somewhere between 10-40 per cent among premature infants.[1] Although these figures do not seem to reflect the incidence reported from certain other countries such as Singapore, Greece and Turkey, nevertheless, it is safe to state that in every country from which incidence figures are available, blood-group incompatibilities

TABLE 1

CAUSES OF EARLY NEONATAL JAUNDICE

I. Mainly indirect bilirubin elevation
   1. Iso-immunization (Rh, ABO, etc.)
   2. Physiologic jaundice (full term and premature)
   3. Bleeding in enclosed spaces-
      a. Hematomas
      b. Intraventricular hemorrhage
   4. Breast-milk inhibition
   5. Respiratory distress syndrome
   6. Hypoxia in infants of diabetic mothers
   7. Familial nonhemolytic jaundice (Crigler-Najjar Syndrome)
   8. Drugs (Novobiocin, Gantrisin, etc.)
   9. Metabolic defects of the red cells (Table 2)

II. Both direct and indirect bilirubin elevation
   1. Infections
      A. Viral
         a. Rubella
         b. Cytomegalic inclusion body disease
         c. Serum hepatitis
         d. Herpes simplex
      B. Spirochetal
         a. Congenital syphilis
      C. Protozoan
         a. Toxoplasmosis
      D. Bacterial
         a. Sepsis
         b. Pyelonephritis
         c. Hepatitis
   2. Galactosemia (mainly direct bilirubin elevation)

TABLE 2

METABOLIC DEFECTS OF THE RED CELLS CAUSING NEONATAL JAUNDICE

I. Early manifestation of inborn errors in red cell metabolism
   1. Glucose-6-phosphate dehydrogenase deficiency
      a. Negroes and Mediterranean Caucasians without chronic anemia
      b. Caucasians with chronic anemia
   2. Pyruvate kinase deficiency
   3. Hereditary spherocytosis
   4. Hereditary elliptocytosis
   5. Triosephosphate isomerase deficiency
   6. 2,3 diphosphoglycerate mutase deficiency
   7. Hereditary stomacytosis
   8. Hereditary absence of glutathione
   9. Glutathione reductase deficiency
   10. Adenosine triphosphatase deficiency

II. Transient defects
   1. Infantile pyknocytosis
   2. Vitamin E deficiency
   3. Heinz body anemia
   4. Drug-induced hemolysis
   5. Hexokinase deficiency (qualitative)

constitute the single major cause of neonatal jaundice. In the countries mentioned, it has been shown that the incidence of unexplained hyperbilirubinemia is quite high. For instance, the recent studies from Ankara have shown that, in as many as fifty per cent of the cases of neonatal hyperbilirubinemia severe enough to require exchange transfusion, no blood-group incompatibilities could be detected nor were there any other known factors to explain the severe jaundice observed (Table 3). Although some of these cases, such as those as those of breast-fed infants with relatively late appearing jaundice, may now be explained on the basis of probable breast-milk inhibition of the bilirubin conjugating system, there still seems to remain a significant number of patients with neonatal jaundice for whom no satisfactory explanation can be found. This was one reason which prompted us* to study the metabolism of the red cells of these patients, since it has been shown that metabolic defects in the red cells may result in premature aging of erythrocytes and, consequently, in hemolysis and jaundice in the newborn. Before discussing the results of our studies briefly, we believe it would be proper to mention a few pertinent facts about red cell metabolism.

The mature red cell is quite different from the rest of the cells of the body since it has no nucleus and is unable divide. To

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maintain its integrity, as well as to perform its functions (e.g. oxygen and carbon dioxide transport), the red cell needs a certain amount of energy. Since, unlike the other cells of the body, it has practically no stored glycogen nor does it have any other carbohydrate store that it can utilize for this purpose, it must have a constant supply of carbohydrates. Genarally, the type of carbohydrate utilized by the red cells is glucose, although other mono or disaccharides may also be used. Almost 90 per cent of the glucose available is utilized via the anaerobic Embden-Meyerhof pathway, while the remaining ten per cent goes through an aerobic breakdown called pentose phosphate pathway (Figure 1). The result of anaerobic utilization is a net gain of two moles of adenosine

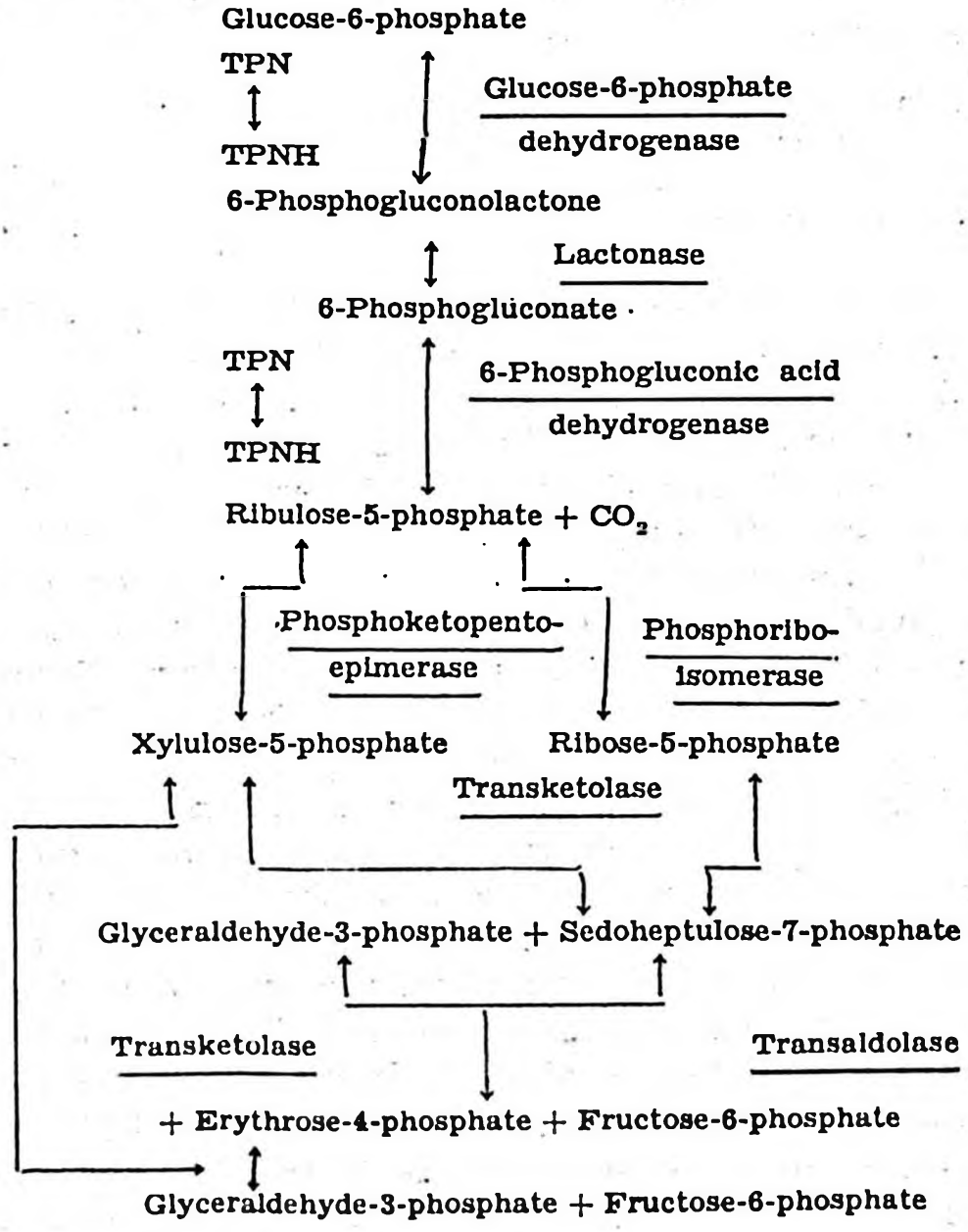

Figure 1. Pentose phosphate pathway.

triphosphate (ATP) per mole of glucose utilized. This energy is used mainly in maintaining the specific internal electrolyte composition of the red cell, as well as for the maintenance of membrane proteins and lipids. Reduced triphosphopyridine nucleotide (TPNH), which is a substance necessary for the protection of hemoglobin from oxidative degradation, is generated by aerobic glycolysis. Many enzymes and co-factors are needed for the proper sequential functioning of both of these pathways. When these metabolic pathways do not function properly, whatever the reason may be, the ultimate result will be the premature aging of the red cell. Finally, it should be mentioned that the newborn's red cells seem to differ in many respects from those of the adult. Needless to say, they contain more fetal hemoglobin, but also other differences have been detected: for instance, they show increared mechanical fragility while their osmotic fragility is actually decreased;[2, 3] they have higher glucose consumption;[4] and, with a few exceptions, all the glycolytic and non-glycolytic enzymes are increased markedly.

An impressive number of red cell metabolic defects have been described recently. As shown in Table 2, those conditions resulting in neonatal jaundice are further divided into two separate categories of defects: a. inherited and permanent; b. transient. Among the conditions listed in the first group, the deficiency of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) is by far the best known. This enzyme, which is inherited as an x-linked trait, was first brought to light during extensive studies on sensitivity to the antimalarial drug primaquine. It was shown that the red cells of these primaquine-sensitive patients had an intrinsic defect rendering them susceptible to hemolysis. It was also found that in almost ten per cent of American Negroes primaquine induced intravascular hemolysis, but this rarely occurred in Caucasians.[5] Further studies showed that the suspected intrinsic defect involved the enzyme G-6-PD.[6] Since then many studies have been done in different parts of the world, revealing that the incidence of this enzyme deficiency was rather high among certain population groups like the Sephardic Jews, Sardinians, Arabs, Greeks, as well as the inhabitants of Southern Europe and the Middle and Near East, such as the Eti Turks.[7, 8] Although this enzyme deficiency may provide some protection against malaria, it brings certain disadvantages to its carriers, especially hemizygous males and homozygous females; for example, favism and hemolytic episodes during certain viral infections, as well as drug-induced hemolytic anemias. Neonatal

hyperbilirubinemia, which was first pointed out in Italian workers [9] and was further supported by extensive studies from Greece,[10] seems to be another disadvantage. At present it is not known why the erythrocytes of susceptible infants should be prone to spontaneous hemolysis as is reported to be the case in affected newborns, although it is postulated by some that either these infants have another yet unknown metabolic defect besides the G-6-PD deficiency [11] or they are actually exposed to unidentified drugs or chemicals which may be extremely hard to detect.[12]

The pyruvate kinase (PK) deficiency is another cause of neonatal jaundice due to an inherited red cell metabolic defect.[13] There are a number of reports indicating that the deficiency of this enzyme may result in quite severe hyperbilirubinemia in the newborn. It must be emphasized, however, that this is not so frequent a cause of neonatal jaundice as is G-6-PD deficiency.

Hereditary spherocytosis, on the other hand, is seen more frequently and recent reports indicate that this condition may manifest itself as neonatal jaundice in as many as 50 per cent of the cases.[14] The actual metabolic defect of the red cells in this hereditary anemia still remains to be discovered, in spite of the fact that numerous studies have been done to solve this mystery. In the newborn, jaundice may be severe enough to require exchange transfusions. Diagnosis of this condition in a jaundiced baby may, at times, be quite difficult since many of these infants may not have an excessive number of spherocytes in their peripheral blood during these hemolytic episodes. Even when present, they could not be taken as a pathognomonic finding, since increased numbers of spherocytes may also be seen in certain other conditions, such as neonatal sepsis and ABO incompatibility. However, the persistence of these characteristic red cells together with increased osmotic fragility beyond the neonatal period may ultimately lead the physician to the correct diagnosis.

Hereditary elliptocytosis is another cause of neonatal jaundice with an unknown metabolic defect of the red cell.[15] The condition is seen much less frequently than hereditary spherocytosis, although it is easily recognized at any age because of the unusual shape of the erythrocytes.

Four other conditions due to red cell enzyme deficiency have been described recently : trisephosphate isomerase deficiency;[16] 2, 3 diphospho-glycerate mutase deficiency;[17] glutathione reductase

deficiency[18] and adenosine triphosphatase deficiency.[19] However, only the first two enzyme deficiencies have been reported so far as a cause of jaundice in the newborn. The reason for the inclusion of the remaining two is the particular nature of their metabolic defect. It seems likely that such cases will be reported in the future. The same probably holds true for the other two conditions listed in Table 2; that is, for hereditary stomacytosis[20] and hereditary absence of glutathione.[21] The list of enzymatic defects leading to hemolytic anemia is quite long and is most likely incomplete at present.

Among the conditions listed in Table 2 as transient defects in red cell metabolism, there are some which do not seem to be at all rare. Infantile pyknocytosis is one of them.[22] This condition manifests itself as a hemolytic anemia limited to the first three months of life and is characterized by the presence of many dense, distorted erythrocytes (pyknocytes). These infants may also have neonatal jaundice. So far, no definite biochemical disturbance could be demonstrated in the erythrocytes of these patients; however, the presence of increased numbers of pyknocytes indicates premature aging of the erythrocytes, which is most likely due to a yet unknown metabolic defect.

Vitamin E deficiency is another condition giving rise to certain metabolic defects in the red cells. It has been found that erythrocytes of vitamin E-deficient patients show increased hemolysis when exposed to H_2O_2. They also seem to have somewhat shortened survival time.[23] Vitamin E apparently acts as an antioxidant, protecting the red blood cell against oxidation. Recently it has also been reported that in some premature infants, the hemolysis and anemia observed may be explained on the basis of vitamin E deficiency.[24] The contribution of this factor to the pathogenesis of prolonged and rather severe neonatal jaundice seen in these small infants has not yet been definitely established. The same holds true for the recent discovery that erythrocyte hexokinase in newborns apparently differs from that of normal adults.[25]

Another interesting condition in this category is congenital Heinz body anemia.[26] Heinz bodies are individual refractile substances probably representing denatured hemoglobin. It is known that red cells of the newborn have a peculiar tendency to form these bodies, a phenomenon which gradually decreases during the first weeks of life. These may also be observed in patients with severe infections or after the administration of excessive amounts of

oxidative drugs such as water-soluble vitamin K. In patients with congenital Heinz body anemia, however, almost every red cell contains one or more Heinz bodies without any demonstrable exogenous influence. The patients have mild anemia and neonatal jaundice is seen quite frequently. The metabolic defect in the red cells of these patients has not so far been clearly defined.

Since Turkey is one of the countries with a rather high incidence of unexplained jaundice, it was decided to study all newborns, full term or premature, having neonatal hyperbilirubinemia. Forty-seven babies were studied, 19 of whom were premature infants (<2500 gm). There were 11 cases (57.9 per cent) of unexplained jaundice among the prematures and 14 (50 per cent) among the full term (Tables 3 and 4). Almost all the etiological possibilities, as listed in Tables

TABLE 3

CAUSES OF HYPERBILIRUBINEMIA IN 28 FULL TERM NEWBORN INFANTS

	Number of Cases	%
Etiology unknown	14	50.00
ABO incompatibility	6	21.42
Rh incompatibility	4	14.30
Sepsis	2	7.14
G-6-PD deficiency	1	3.57
Hereditary spherocytosis	1	3.57
Total	28	100.00

TABLE 4

CAUSES OF HYPERBILIRUBINEMIA IN 19 PREMATURE INFANTS

	Number of Cases	%
Etiology unknown	11	57.90
ABO incompatibility	3	15.80
Rh incompatibility	2	10.52
Sepsis	2	10.52
G-6-PD deficiency	1	5.26
Total	19	100.00

1 and 2, were explored and many hematological and biochemical studies were undertaken. Five normal full term infants were also studied as controls. The results of these studies revealed that in a significant percentage of patients no etiological factor could be demonstrated. Most of the cases of jaundice seen in the premature

infants, however, could be explained on the basis of immaturity of the hepatic enzymes. The possibility of the inhibition of the glucuronizing system by breast milk could not definitely be excluded in the full term babies since all of these except five were on breast feeding. Among the red cell enzymes, G-6-PD, pyruvate kinase, lactic dehydrogenase and acetylcholine esterase levels were determined. Two patients with G-6-PD deficiency were found. Acetylcholine esterase levels were low, as expected in almost every case of ABO incompatibility. Detailed results of this study will be published later. However, it must be emphasized again that neonatal jaundice of unexplained etiology still remains an important, unsolved medical problem for certain countries and requires more extensive metabolic studies.

Summary

The place of erythrocyte enzymes in the etiology of neonatal jaundice is briefly reviewed. The results of a recent study on infants with unexplained jaundice is presented.

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