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HEMOLYTIC ANEMIA IN DISORDERS OF RED CELL METABOLISM

Ernest Beutler, M.D.

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Series Editor: Maxwell M. Wintrobe, M.D.
University of Utah, Salt Lake City

THE RESPIRATORY FUNCTIONS OF BLOOD
Lars Garby, M.D. and Jerry Meldon, M.D.

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Ernest Beutler, M.D.

TRACE ELEMENTS AND IRON IN HUMAN METABOLISM
Ananda S. Prasad, M.D.

FOREWORD

I am prepared to predict that this monograph by Dr. Ernest Beutler will long serve as a model for monographs dealing with topics in medical science. I make this bold statement because we encounter in this work a degree of accuracy and authoritativeness well beyond that found in much of the medical literature. Too often, a monograph is simply a review of past reviews. The preparation of an exhaustive and completely accurate study such as the present one is a very laborious task; consequently, many authors make extensive use of the reviews of earlier writers assuming that the latter have checked and evaluated each previously published report. Unfortunately, however, this assumption of validity has not always been correct.

Dr. Beutler, who is a world authority on the subject about which he writes, was determined to make this book as correct and complete as possible, and, to this end, has checked all the original sources. Nowhere else will such an exhaustive bibliography be found. Moreover, he has also undertaken to reevaluate in the light of current knowledge material published in earlier days. This he is eminently able to do, and in some instances his investigations have resulted in new interpretations. The result is a volume that will be recognized as truly the last word on this important subject.

Maxwell M. Wintrobe

Salt Lake City

PREFACE

Why another review of red cell enzyme defects associated with hemolytic anemia?

A good question, and one that I asked myself many times in planning and in writing this book. The fact that Professor Maxwell Wintrobe asked me to prepare this volume was the initial stimulus for the project. A request from Dr. Wintrobe is in itself very persuasive, but neither he nor I would have wanted this book to represent a mere reprise of what has been written before.

What, then, is new and what is different about this book?

First of all, most reviews of red cell enzyme deficiencies, particularly those dealing with G-6-PD, have relied on the accuracy of previous reviews for many of the data which they contained. This has had predictable, undesirable effects. Inadvertent errors, including some from my own early reviews, have been perpetuated in review after review. Moreover, the earlier data have never been carefully reevaluated in the light of current knowledge. Clearly, what was needed was a review of all of the original reports, regardless of obscurity of journal or strangeness of language. For example, the list of drugs to which G-6-PD-deficient persons are sensitive is published and republished, occasionally with the addition of new drugs. Seemingly, no drugs are ever deleted, for the original data seem never to be reevaluated. Persons with G-6-PD A− detected in population surveys are given cards which warn them not to take aspirin, Gantrisin, or chloroquine (none of which are hemolytic), but they are not counseled against the ingestion of hemolytic drugs such as Furadantin and Bactrim. Similar misinformation serves as a basis of "roundsmanship" as attending physicians display their knowledge of literature by warning house officers against the administration of drugs which are in reality innocuous to G-6-PD-deficient individuals. It seemed to me that a critical reappraisal of the drugs which produce hemolysis in G-6-PD deficiency was sorely needed. Many of the drugs on the list which is published, circulated, and republished were implicated before it was recog-

nized that infection was a common precipitating cause of hemolysis in G-6-PD deficiency.

The inaccurate list of drugs which putatively produce hemolysis in G-6-PD deficiency is only one of many misconceptions which have been reiterated in the literature. Other common erroneous ideas examined in this book include the putative undesirability of the use of G-6-PD-deficient blood for transfusions, the etiologic role of glutathione reductase deficiency in a variety of diseases, and that of glutathione peroxidase, enolase, ATPase, and phosphogluconate dehydrogenase deficiency in hemolytic anemia.

There are data in this book which should be of value to the student of red cell abnormalities. A comprehensive review of the incidence of G-6-PD deficiency among various populations has not been published for many years. An up-to-date, carefully edited listing of properties of G-6-PD variants is also provided.

Although the biochemical details of the various types of enzyme abnormalities are of importance, and are given generous coverage in this book, I have tried to give primary emphasis to the clinical manifestations of the red cell enzyme defects. For example, a review of the effect of splenectomy in the various types of enzyme defects may guide the clinician who is charged with the responsibility for the care of one of these patients. In order to provide comprehensive coverage of some topics, it has been necessary to omit coverage of others. I have therefore reviewed only those red cell enzyme defects which are known to produce significant shortening of the red cell life span and those which have been alleged to do so. Various interesting and important red cell enzyme deficiencies are excluded. I have not attempted to discuss defects such as acatalasemia, galactose-1-phosphate uridyl transferase deficiency, galactokinase deficiency, adenosine deaminase deficiency, ITPase deficiency, phosphoglucomutase deficiency, UDP glucose-4-epimerase deficiency, lactate dehydrogenase deficiency, NADPH diaphorase deficiency, or acetylcholinesterase deficiency, because they are not associated with significant hemolytic consequences. Neither have important defects such as hereditary spherocytosis, elliptocytosis, or stomatocytosis been included; the enzymatic basis of these abnormalities is not known.

Preparation of this book, particularly with the necessity for consulting all of the primary references, was a difficult and time-consuming task which could not have been achieved without loyal assistance from both my staff and my family. Mrs. Janet Manning, Marianne Moss, and Sharyn Webb provided the secretarial assistance without which this work would have been impossible. Mr. John Carrigan, City of Hope librarian, aided me in a preliminary review of the literature concerning drug-induced he-

molytic anemia and in making available to me obscure journals from obscure places. Mrs. Florinda Matsumoto aided me in the preparation of the table of G-6-PD variants. I am grateful to Dr. Akira Yoshida and Dr. Karl G. Blume for their critical reviews of the sections concerning G-6-PD and pyruvate kinase, respectively. My sons, Steve and Bruce, reviewed clinical reports on pyruvate kinase deficiency and data on incidence of G-6-PD deficiency, respectively. My wife, Bonnie, provided skillful editorial assistance and literary polish.

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Ernest Beutler

Duarte

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1

THE RED CELL

Evolution from single-celled organisms to complex metazoa requires the development of systems for the distribution of nutrients and oxygen to all cells of the body. The need to supply oxygen molecules reliably, consistently, and in large amounts has been met by different animals in different ways, but the most efficient system is one which has been adopted by all vertebrates. It consists of a specialized cell, the erythrocyte, filled with a protein uniquely suited to this task, hemoglobin.

By its very nature, specialization implies emphasis of certain attributes. Performance of its specialized function of oxygen transport required that the human erythrocyte evolve with the following characteristics: (1) the capacity to synthesize a high concentration of the respiratory pigment, hemoglobin; (2) the ability to maintain the integrity and function of the hemoglobin during its life span in the circulation; (3) a shape which allows efficient oxygen delivery to the tissues; and (4) the capacity to move rapidly through all areas of the body without loss of its contents. The development of these characteristics occurred at the expense of others; many of the metabolic functions normally carried out by most body cells, such as the citric acid cycle, have been sacrificed.

1.1. Red Cell Structure

The red cell has a structureless, relatively homogeneous interior bounded by a highly structured membrane at its interface with the plasma or other suspending medium. The interior of the cell consists of a highly concentrated aqueous solution of hemoglobin containing most of the glycolytic enzymes, nucleotides, 2,3-diphosphoglycerate, and other phosphorylated metabolic intermediates, glutathione, and electrolytes. The red cell, in contrast to the plasma, has a high potassium and low sodium content.

The red cell membrane is now generally regarded as a complex lipid bilayer. The outer and inner layers contain the hydrophilic phosphate groups of phospholipids, with the hydrophobic fatty acid chains pointing inward. The outer leaflet is particularly rich in phosphatidylcholine and sphingomyelin, while the inner leaflet contains more of the phosphatidylserine.[1] Cholesterol molecules are scattered throughout the outer leaflet so that it contains approximately one molecule of cholesterol for each molecule of phospholipid. The membrane is composed not only of lipids but also of a large number of different proteins. One of these, designated *spectrin*, seems to form a network lining the inner surface of the membrane.[2,3] An actin-like protein which may interact with spectrin has also been detected.[4] The glycoprotein, *glycophorin*, appears to pass through the entire lipid bilayer, being exposed both at the outer and inner aspects of the membrane. This substance has been studied in considerable detail, and its amino acid composition has also been studied.[5] A hydrophobic region is present in the central portion, the location of the membrane fatty acid chains; glycophorin is more hydrophilic at the outer and inner aspects of the membrane where it comes into contact with the plasma and with the red cell contents, respectively. Other proteins may also pass through the entire membrane, be exposed only at the outer or the inner portion, or lie entirely submerged within the lipid bilayer. Some enzymes, e.g., acetylcholinesterase, NADPase, ATPase, and glyceraldehyde phosphate dehydrogenase (GAPD), appear to be firmly membrane bound.

The red cell membrane exhibits a highly selective permeability to various ions. Anions, such as chloride and bicarbonate, pass through the membrane extremely rapidly, and equilibrate according to the concentrations predicted by the Donnan membrane equilibrium. In contrast, the membrane is quite impermeable to cations, such as sodium and potassium.[6] A marked gradient exists between the concentration of potassium, which is approximately 90 mM within the cell and 4 mM in the plasma, and that of sodium, which is 8 mM within the cell and 140 mM in the plasma. Virtually no calcium gains access to the interior of the cell, but small quantities are present in the membrane. The amount of calcium in the membrane seems to be related to its flexibility.[7,8]

The resting human erythrocyte has a biconcave shape. It is clear that this shape is a function of the membrane rather than of the contents of the cell, since red cell ghosts are also biconcave. Furthermore, the location of various parts of the membrane with respect to the form of the cell does not appear to be fixed: a part of the membrane which is at the center of the biconcavity at one moment may be on the rim at another.[9] Under physiologic conditions the cell is rarely in an unstressed state. Continually buffeted by strong shear stresses in the circulation, its shape is distorted.

The normal human erythrocyte is remarkably deformable. It can be pulled into a long, fiber-like structure whose length is many times the diameter of the normal cell. It can assume dumbbell shapes to facilitate its passage through narrow apertures such as those of the spleen. It may fold over bifurcations in blood vessels, forming two greatly elongated teardrops until slipping down one channel or the other intact. It may twist and turn and flex to assume a great variety of other forms. The great deformability of the erythrocyte is not due to the membrane's capacity to stretch; in reality the membrane can tolerate very little stretch without rupturing. Rather, the red cell membrane offers very little resistance to bending. Since its surface area is considerably larger than that needed to enclose the contents, considerable distortion is possible without appreciable stretch. It is generally believed that loss of flexibility of the membrane may be a common denominator in the origin of many different types of hemolytic anemia.[10,11]

1.2. Red Cell Metabolism

The mature red cell of the human has been able to sacrifice many of the metabolic functions required for the existence of most other body cells. It has no nucleus or ribosomal apparatus, and hence cannot synthesize protein. It has lost its mitochondria, and therefore cannot metabolize pyruvate through the citric acid cycle. It cannot carry out *de novo* synthesis of nucleic acids or lipids. Yet the red cell is metabolically active, and loss of these functions has not compromised its ability to survive in the circulation or to carry out its major function of oxygen transport. Although it is sufficiently versatile to extract energy from a number of different substrates, its principal energy source normally is glucose. Deprived of glucose, the erythrocyte cannot function and survive. It cannot maintain the gradient of sodium and potassium which exists across the normal red cell membrane. Neither can it prevent the accumulation of calcium in the red cell membrane. Methemoglobin and oxidized glutathione accumulate, especially in the presence of oxidative stresses. The energy-deprived erythrocyte becomes echinocytic, then spherocytic, and ultimately undergoes osmotic lysis.

The red cell metabolizes glucose through two main routes, the Embden–Meyerhoff pathway, and the hexose monophosphate pathway (Table I). In the Embden–Meyerhoff pathway, glucose is catabolized to pyruvate or to lactate. A major portion of the energy derived from the glucose molecule is stored in the high energy phosphate of adenosine triphosphate (ATP). Reducing power is generated in the conversion of NAD^+ to NADH, the form of the coenzyme which reduces methemoglobin to he-

TABLE I
The Functions of the Two Main Pathways of Glucose Metabolism in the Erythrocyte

Functions of the Embden–Meyerhoff pathway (glucose-6-phosphate ⟶ lactate)	Functions of the hexose monophosphate pathway (glucose-6-phosphate ⟶ CO_2 + pentose, triose, etc.)
ADP ⟶ ATP (pumps Na^+ and K^+)	
NAD^+ ⟶ NADH (reduces methemoglobin)	$NADP^+$ ⟶ NADPH (reduces GSSG and protein–SG disulfides)
1,3-DPG ⟶ 2,3-DPG (regulates oxygen dissociation curve)	HEXOSE ⟶ PENTOSE (provides substrate for nucleotide synthesis)

moglobin. 2,3-Diphosphoglycerate (2,3-DPG), an important modulator of hemoglobin oxygen affinity, is also synthesized in this pathway. The hexose monophosphate pathway (HMP) accounts only for approximately 10% of glucose metabolism in the resting cell. The rate of metabolism through the HMP is normally controlled by the availability of $NADP^+$. Under oxidative stress, therefore, when NADPH is oxidized to $NADP^+$, a much larger proportion of the total glucose metabolized may flow through this pathway. The principal function of the HMP is to maintain $NADP^+$ in its reduced form, NADPH. This coenzyme is required to maintain glutathione in the reduced form, a reaction which is important in protecting the erythrocyte from peroxidative damage. In reducing $NADP^+$ to NADPH the first carbon of glucose is oxidized to carbon dioxide, and a pentose is formed. This 5-carbon sugar may be used in the synthesis of nucleotides by the erythrocyte, or may undergo molecular rearrangements which result in further metabolism of the 3- and 6-carbon sugars formed in the Embden–Meyerhoff pathway. Thus, whereas ATP and 2,3-DPG are not formed in the hexose monophosphate pathway itself, glucose passing through the hexose monophosphate pathway may participate in the formation of these substances after the direct oxidative pathway has been traversed.

1.2.1. Embden–Meyerhoff Pathway (Fig. 1)

Glucose enters the red cell rapidly through an unidentified carrier in the membrane.[12,13] Entry of glucose into erythrocytes is temperature sensitive[14] but not insulin dependent.[15] In contrast to that in most tissue cells,

the glucose concentration within the red cell water is the same as that in plasma water. Entry of glucose into red cells never appears to be a limiting factor in its utilization.

The first step in the utilization of glucose is its phosphorylation by hexokinase. ATP serves as the phosphate donor, and magnesium is required. Some of the properties of human red cell hexokinase are summarized in Table II. Hexokinase deficiency is a rare cause of hereditary nonspherocytic hemolytic anemia (NSHA; see Chapter 4).

The product of the hexokinase reaction, glucose-6-phosphate, is in equilibrium with glucose-1-phosphate through phosphoglucomutase and glucose-1,6-diphosphate, and with fructose-6-phosphate through glucose phosphate isomerase (GPI; see Table II). It is the latter reaction which is

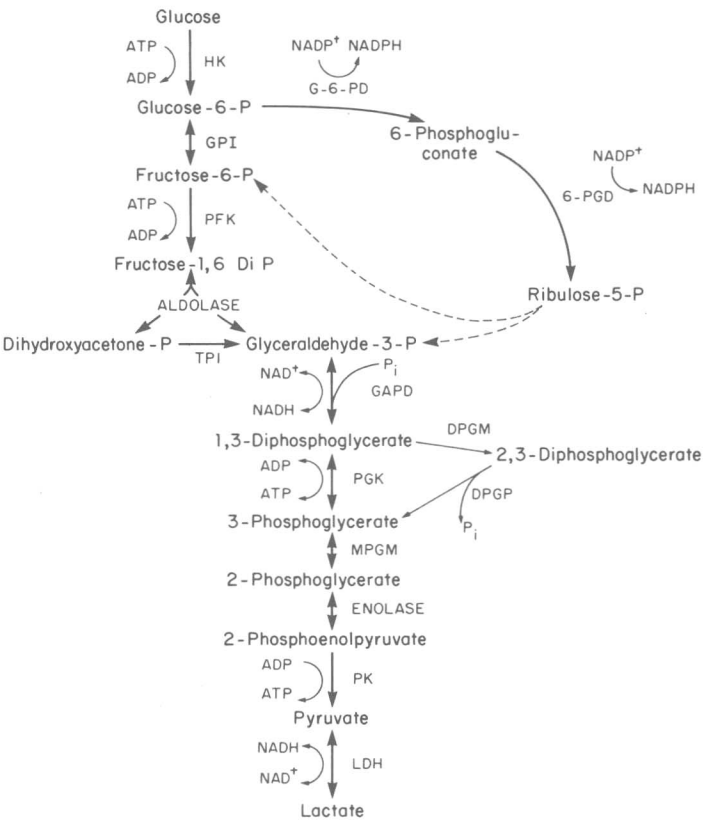

FIGURE 1. Major pathways of red cell metabolism. The abbreviations are defined in the index.