DISORDERS OF HEMOGLOBIN

Genetics, Pathophysiology, and Clinical Management

SECOND EDITION

This book is a completely revised new edition of the definitive reference on disorders of hemoglobin. Authored by world-renowned experts, the book focuses on basic science aspects and clinical features of hemoglobinopathies, covering diagnosis, treatment, and future applications of current research. While the second edition continues to address the important molecular, cellular, and genetic components, coverage of clinical issues has been significantly expanded, and there is more practical emphasis on diagnosis and management throughout.

The book opens with a review of the scientific underpinnings. Pathophysiology of common hemoglobin disorders is discussed next in an entirely new section devoted to vascular biology, the erythrocyte membrane, nitric oxide biology, and hemolysis. Four sections deal with α and β thalassemia, sickle cell disease, and related conditions, followed by special topics. The second edition concludes with current and developing approaches to treatment, incorporating new agents for iron chelation, methods to induce fetal hemoglobin production, novel treatment approaches, stem cell transplantation, and progress in gene therapy.

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Bernard G. Forget is a distinguished physician scientist in Hematology, nationally and internationally recognized for research accomplishments in the field of Molecular Hematology pertaining to the molecular biology of gene expression in blood cells and the molecular basis of hereditary disorders of the red blood cell, including hemoglobinopathies. He is the co-author with Dr. H. F. Bunn of a highly respected textbook entitled Hemoglobin: Molecular, Genetic and Clinical Aspects, (WB Saunders Co., Philadelphia, 1986). He is the senior author of a large number of scientific publications in the field of Molecular Hematology and red blood cell disorders, published in leading journals.

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SECOND EDITION

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Foreword

H. Franklin Bunn

The study of hemoglobin continues to be a rewarding endeavor. Cumulative progress since the turn of the last century has laid cornerstones in protein chemistry and molecular genetics and has provided a wealth of insight into the pathogenesis of some of the world’s most prevalent and devastating disorders. The first edition of Disorders of Hemoglobin, published 8 years ago, was a comprehensive compilation and analysis of the basic science of hemoglobin and its application to the thalassemias, sickle cell disease, and other globin mutants that spawned a wide range of clinical phenotypes. This second edition now presents an updated overview of all aspects of the hemoglobin story as well as a detailed account of the impressive advances that have been made in biochemistry, genetics, and clinical investigation.

Hemoglobin boasts a proud history. By the end of the nineteenth century, it was well established that hemoglobin was a composite of protein and heme that could reversibly bind oxygen and that this substance was found in almost all living creatures. Entry into the twentieth century marked the dawn of quantitative physiology, biochemistry, and the application of the scientific method to medicine. All three of these developing disciplines owe their early impetus to hemoglobin and the lessons learned from this remarkable molecule. Physiologists from Scandinavia (Bohr and Krogh) and England (Barcroft, the Haldanes, and Roughton) made accurate equilibrium and kinetic measurements of oxygen–hemoglobin binding as a function of pH and thereby provided a mechanistic understanding of the reciprocal transport of oxygen from lung to tissues and of acid waste from tissues to lung. These early contributions set the stage for an appreciation of how the homeostasis of the organism depends on the orderly integration of its organ systems.

The fledgling science of biochemistry was given a jump start by the studies of Adair and Svedberg, which established that hemoglobin is a uniform protein with a large but narrowly defined molecular weight and was therefore, like sodium chloride and glucose, a bona fide molecule. Hemoglobin and its cousin myoglobin were the first proteins whose structures were solved at high resolution by X-ray crystallography by Perutz and Kendrew, respectively, thereby, providing an opportunity for detailed exploration of structure–function relationships. Hemoglobin was the first multisubunit protein to be understood at the molecular level and therefore was the model system used by Monod, Changeux, and Wyman for establishing the principles of allostery, which dictate the regulation of a broad range of enzymes, receptors, transcription factors, and so on.

The linkage of specific diseases to abnormalities of specific molecules began with Pauling’s demonstration in 1949 that patients with sickle cells have hemoglobin with an altered surface charge. Within 8 years, Ingram demonstrated that sickle hemoglobin differs from normal hemoglobin only by a substitution of valine for glutamic acid in the sixth residue of the β-globin subunit. This was the first example of how an abnormal gene can change the structure of a protein and, therefore, verified in a most satisfying way the Beadle–Tatum one gene–one enzyme hypothesis.

During the last quarter of the twentieth century, with the development of recombinant DNA technology and genomics, hemoglobin again became primus inter pares among biological molecules. Indeed, the human globin genes were among the first to be molecularly cloned and sequenced. This soon led to the identification of a wide range of globin gene mutants responsible for the α and β thalassemias. Understanding the mechanisms by which these genotypes impair globin biosynthesis provided insight into the diverse clinical manifestations encountered in patients with different types of thalassemia. In addition, the evolving knowledge of human globin genes enabled the development of molecular techniques for antenatal diagnosis and polymorphism-based population studies, both of which were then applied to many other disorders.

To date, more than 1,000 hemoglobin variants have been discovered and characterized. Study of these variants, so amply documented in this book, established the principle of how a mutant genotype alters the function of the protein it encodes, which in turn can lead to a distinct clinical phenotype. This linkage is at the heart of how molecular genetics impacts our understanding of pathophysiological mechanisms.

Thus, hemoglobin held center stage in the biomedical discoveries of the twentieth century, and, in the new millennium, there is no indication that the pace has slackened. This book begins with authoritative and up-to-date coverage of all aspects of hemoglobin, beginning with overviews of erythropoiesis, globin gene regulation, and structure–function relationships. Subsequent sections of the book are devoted to in-depth coverage of the thalassemias, sickle cell disease, and other hemoglobinopathies. A recurrent theme is how understanding pathophysiology at the molecular
level has informed the design and development of novel, rationally based therapy.

This second edition incorporates a number of advances that have been made in the past 8 years. Chapter 4 describes the important insights that have accrued from the discovery of α-hemoglobin stabilizing protein (AHSP), the chaperone that protects the α-hemoglobin subunit during assembly of the tetramer. Chapters 6, 10, and 11 include new information on nitric oxide and its controversial roles in allosteric modulation of hemoglobin function and in the pathophysiology of sickle cell disease and other types of hemolytic anemia. Chapter 27 presents recent information on the contribution of genetic polymorphisms to the clinical phenotypes of sickle cell disease and thalassemia. The last 4 chapters cover the development of oral iron chelators as well as bolder therapeutic strategies, including impressive progress in globin gene therapy.

The creative energy that continues to bear down on all aspects of hemoglobin research is well represented by the impressive list of basic and clinical investigators who have contributed to this book. In any field at the cutting edge of science, controversies enrich the scientific dialogue among hemoglobinologists. In carefully reading chapters on closely related topics, the thoughtful reader will adopt a policy of caveat emptor, appreciating that strongly held opinions need to be vetted by both experimentation and alternative hypotheses. This proviso notwithstanding, Disorders of Hemoglobin offers authoritative and comprehensive coverage of one of the most exciting and fruitful areas at the interface of bioscience and clinical medicine.
Preface

Eight years have passed since this monograph first appeared, and the advances in basic, translational, and clinical research during this interval justify a new edition. To conserve space and avoid duplicating our first edition, we review very briefly historical aspects, summarize established older information, and focus on the progress of the past 8 years. Although some older references are retained, we have tried to focus on the literature since 2001. In expanding our coverage of clinical issues, we also have decreased the length of the book by considering together pathophysiological features common to many hemoglobin disorders such as vasculopathy, erythrocyte membrane damage, and mechanisms of hemolysis. More than half of the contributors to this volume are either new authors or previous authors addressing different topics; David Weatherall has joined the editorial team.

Hemoglobin has been an interest of basic and translational scientists, clinicians, and clinical diagnostic laboratories. So, we continue to address the molecular, cellular, genetic, diagnostic, and clinical aspects of hemoglobin disorders. When applicable, we provide practical recommendations for diagnosis and treatment. The first section of the book again focuses on molecular, cellular, and genetic aspects of hemoglobin and includes discussions of developmental hematopoiesis, erythropoiesis, globin genes and their regulation, minor normal hemoglobins, and an update on new structural and functional features of normal and variant hemoglobins. Pathophysiology of hemoglobin disorders follows, with new chapters on vascular biology, the erythrocyte membrane, the biology of nitric oxide, mechanisms of hemolysis, and how animal models of disease provide new pathophysiological insights. Four sections deal with diagnosis, complications, and treatment of α thalassemia, β thalassemia, and related conditions, including hemoglobin E diseases, sickle cell disease, and less common genetic and acquired hemoglobin disorders. This is followed by special topics such as population genetics and the health burden of hemoglobin disorders, the genetic modulation of sickle cell disease and thalassemia, and developments in laboratory detection, including antenatal diagnosis. Finally, current and developing approaches to treatment, incorporating new agents for iron chelation, methods to induce fetal hemoglobin production, novel treatment approaches such as antioxidants, antiinflammatory agents, enhancement of nitric oxide effects, and agents that modulate membrane cation and water transport are discussed, concluding with the use of stem cell transplantation and progress in gene therapy.

Ronald L. Nagel (pictured), a coeditor of the first edition, has retired as Irving D. Karpas Professor of Medicine, Physiology and Biophysics and Head of the Division of Hematology at Albert Einstein College of Medicine. Although no longer a coeditor of this monograph, his influence in the field is felt in most chapters. His contributions to the structure, function, pathophysiology, and genetics of hemoglobin disorders are vast and time tested. The editors, and the field of hematology, will miss his scientific insight and originality.

The Editors
Introduction

David J. Weatherall

A few years ago, an eminent British professor of medicine, while reviewing a new edition of a well-known textbook of medicine, suggested that works of this type were becoming valueless because they were already out of date by the time they were published. His derogatory comments went further: Having taken the trouble to weigh the book, he suggested that volumes of this type would suffer the same fate as dinosaurs and become extinct by collapsing under their excessive weight. Even allowing for this bizarre and completely erroneous view of the biological fate of the dinosaurs, does this argument carry any weight beyond its metaphorical context?

Undoubtedly, there is feeling rife among medical publishers that the day of the major monograph in the biological sciences may be coming to an end. They argue that there is so much information online that the need for works of this type is becoming increasingly limited. Is this really the case? Although it is impossible to deny that the long gestation of monographs of this type may lead to the omission of the occasional “breakthrough” in a field, it seems very important that in any rapidly moving area of the biomedical sciences there is a regular and broad critical review of where it has got to and how it has been modified by recent advances. Not uncommonly in medical research and practice, today’s breakthrough is tomorrow’s breakdown.

Is the hemoglobin field moving rapidly? This was another question that had to be considered by the editors of this new edition. As judged by the amount of space given to disorders of the red cell in current journals, the volume of work in this field seems to have declined considerably over recent years. A visitor from outer space, browsing through the journals, might be excused for wondering how Homo sapiens transfers oxygen to their tissues. Hence, it might have been perceived that there is insufficient material to warrant this new edition.

A broader review of the field over recent years suggests, however, that this is not the case. There undoubtedly have been major advances in our understanding of the regulation of hematopoiesis, some of which have important implications for a better understanding of the pathophysiology of the hemoglobin disorders that may, in the longer term, lead to more definitive approaches to their management. Furthermore, there have also been dramatic developments in many areas of genome technology that have direct application to the many unanswered questions of the hemoglobin field, not in the least the reasons for the remarkable phenotypic variation of its diseases. Of even greater importance, there has been a genuine increase in the appreciation of the major public health burden that these diseases are likely to cause in the future. This is particularly relevant to the poorer countries of the world in which the epidemiological transition following improvements in nutrition and basic public health is resulting in a reduction in neonatal and childhood mortality; many babies with severe hemoglobin disorders who would previously have died in early life are now surviving to present for diagnosis and management.

It is only in the last few years that these public health issues have been recognized by the major international health agencies. In 2002, the World Health Organization (WHO) published a report, *Genomics and World Health*, in which the hemoglobin disorders were described as a prime example of how the new technology of molecular genetics can be applied for the benefit of poorer countries. At the 118th session of the WHO Executive Board, held in 2006, the sickle cell disorders and thalassemias were formally recognized as major health burdens that required immediate action. In 2007, it was decided to include the hemoglobin disorders in the Global Burden of Disease Program, an international study conducted under the auspices of several universities, the WHO, the Bill and Melinda Gates Foundation, the World Bank, and others that attempts to define the relative global burden posed by each of the major diseases. Previous versions of this work have undoubtedly had a major influence in developing healthcare policies by governments and international healthcare agencies.

Clearly, this new edition is appearing at the same time as a major drive to define the most appropriate ways of controlling and managing the hemoglobin disorders, particularly in the developing countries, and to determine the most cost-effective and efficient ways of approaching this problem. We hope, therefore, that this updated distillation of knowledge about the scientific, clinical, and epidemiological aspects of this field will be of value to scientists and clinicians, not only to those in wealthier countries but particularly to those who are attempting to cope with these diseases with limited resources in the developing countries of the world.

There is also an important message for our younger readers. There are still some extraordinarily exciting areas of this field to be pursued, not in the least a better understanding of the reasons for the remarkable clinical
diversity of all the hemoglobin disorders; a better appreciation of their pathophysiology at the molecular level with respect to novel approaches for their more definitive management; and an understanding of the long-neglected role of the environment in their clinical diversity, the cellular mechanisms whereby protection against malaria has resulted in their extremely high frequency, how current knowledge of their diagnosis and control may be applied in the poorer countries of the world, and many other stimulating questions. Currently, the hemoglobin field offers challenges ranging from basic cell and molecular biology through clinical research at the bedside to epidemiology, public health, and the social sciences.

Finally, we thank Cambridge University Press and particularly Beth Barry and more recently Larry Fox for continued support of this project. We are also extremely grateful to the authors from many parts of the world who have willingly given their time to writing parts of this new edition, and for the personal help that we have received from Liz Rose, during its preparation. It is particularly gratifying to be able to report that the marriages of the four editors have survived another edition.