
Preface

Hemoglobin and Hemoglobinologists

This volume, *Hemoglobin Disorders: Molecular Methods and Protocols*, will be introduced with a review of the great milestones in the field, and the scientists responsible for those achievements. The history of hemoglobin can be divided into three periods: the Classical period, the Modern period, and the Post-Modern period.

I am inclined to include as the four major members of the classical period Francis Roughton, Quentin Gibson, Jeffries Wyman, and Linus Pauling, not only because of their achievements, but also because of the superb scientists they trained and/or influenced.

Francis John Worsely Roughton (1899–1972) (**Fig. 1**), in his laboratory at Trinity College in Cambridge, England, made the first measurements of the rapid reaction of oxygen with hemoglobin at the millisecond scale, at first by flow-mixing methods and later by flash photolysis. He not only opened an era of molecular research of hemoglobin, but also invented the methodology for fast reactions through the use of laser technology, which was later improved by others so that even faster reactions could be detected. Another contribution of Roughton was the education of Quentin H. Gibson (**Fig. 2**), his favorite student, who, in his laboratory in Sheffield, continued to expand the horizon of ligand binding to hemoglobin, defining the oxygen binding constants for each of the hemes of hemoglobin. Though this did not, as expected, solve the underlying mechanism of ligand cooperativity as discussed below, it was nonetheless an important milestone.

Roughton would later have a surprising influence in the Italian hemoglobin group because he trained Luigi Rossi-Bernardi, and because Quentin Gibson introduced Jeffries Wyman to Eraldo Antonini, the hemoglobin man in Rome in that period (**I**). In a meeting in Bellagio, Lake Como, Luigi regaled us with stories about this highly talented and very eccentric investigator. It was fortunate to science that eccentricity was perfectly acceptable in England, unlike in other places in the world.

Finally, Quentin continued his highly productive career after emigrating to the United States in the early 1960s, working independently first in Britton Chance's lab in Philadelphia and then at Cornell, where he trained John Olson, who brilliantly carried on the torch and is an author in this book. I consider Quentin my mentor, along with Helen Ranney. It was most exciting to solve the molecular basis of the hemoglobin/haptoglobin reaction together.



Fig. 1. Francis John Worsely Roughton

Quentin Gibson, who is an MD, was famous for having a lathe in the middle of the lab, useful for tinkering with homemade instrumentation. This wonderful “tinkering” habit of British scientists came in handy during World War II, to the benefit of the world. Gibson is still scientifically active, and has contributed widely to the hemoglobin ligand binding field (see Chapter 5). He also wrote his recollections of the life and work of Francis J. Roughton in 1973, after Roughton’s death at the age of 73 years (2).



Fig. 2. Quentin H. Gibson

Jeffries Wyman (1901–1995) (**Fig. 3**) was a Boston Brahmin and a remarkable American biophysicist whose grandfather was one of the founders of the National Academy of Science. A Harvard man, he developed an interest in proteins and in 1937 wrote his first hemoglobin paper on the pH titration curves, or oxy-deoxy-hemoglobin (3). He exhibited a unique understanding of thermodynamics in his analysis of linked function reciprocal relations (1948). He later came back to this subject with a landmark book, *Binding and Linkage: Functional*

Chemistry of Biological Macromolecules, coauthored with Stanley J. Gill, who derived great comfort from this enterprise in the last years of his life.

According to Edsall (4), Wyman, while visiting colleagues in Japan in 1950, had an insight based on the work of Felix Haurowitz, a New York scientist who used to walk about with a vial of growing hemoglobin crystals in his vest pocket, so as to maintain the solution close to 37°C. Haurowitz did a remarkable and simple experiment: he reduced a crystal of oxyhemoglobin with dithionite and observed its breakage and dissolution. He concluded that these two ligand states of hemoglobin had different crystal habits. Wyman, in turn, concluded that the result was the consequence of hemoglobin in two different conformational states: in oxy (met in reality) and deoxy, a remarkable anticipation of Perutz's work.

Wyman's wanderlust took him to the four corners of the world. After the death of his first wife, he left Harvard and the United States for Paris, where he was the first Cultural Attache to the American Embassy. After that, it was an International Organization job in Egypt, and then escapes to the Congo, Alaska, Papua, New Guinea, and so forth. But his most important visit by far was to Rome, where he became part of the hemoglobin team lead by Eraldo Antonini (1). Italy became his home for most of his life, in spite of the fact that he never obtained a permanent status, and needed to go to Switzerland every year to renew his visa. He never learned to speak Italian.

The Rome group, integrated by Maurizio Brunori, Emilia Chiancone, and others, became a strong presence in the field, concentrating on the biophysical and biochemical aspects of hemoglobin. Another participant in this interactive hemoglobin world was Quentin Gibson, who collaborated with Eraldo early on and had to carry instrumentation and glass artifacts through the corridors and yards of the University of Rome, because it was unseemly for an Italian professor to do so. Maurizio, of course, became the leader of this highly productive group after the untimely death of Eraldo at the age of 52, keeping the high standards set by its founder. During my first visit to Rome, Maurizio introduced me to Wyman, and like everybody else, I was in awe of the magnetic field of his mind and his ability to contribute brilliantly to any problem that might be presented to him.

Finally, the emergence of the Jacob-Monod-Changeux allosteric model fit Wyman's insight into the workings of hemoglobin and rapidly adopted its

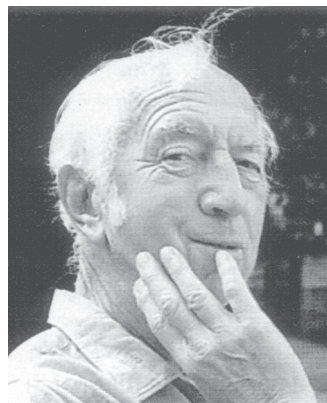


Fig. 3. Jeffries Wyman

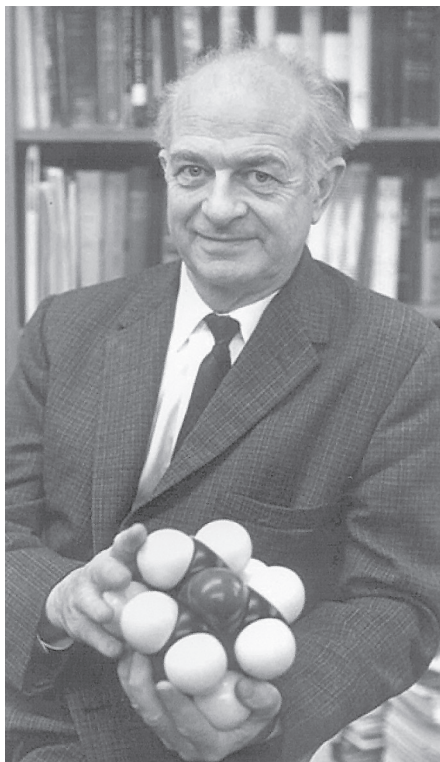


Fig. 4. Linus Pauling

nomenclature. On the other the hand, Eraldo Antonini resisted this concept, postulating an alternative dimer-based model. The demonstration that haptoglobin binds exclusively hemoglobin dimers—and does not bind deoxy-hemoglobin (5,6) because it does not dimerize—made this proposal untenable.

The final member of the Classical period was Linus Pauling (1901–1994) (**Fig. 4**), a double Nobel Prize winner, and another eccentric and brilliant scientist. He should be considered a luminary in the field of hemoglobin for two reasons. First, he proposed (and demonstrated) that a hemoglobin abnormality had to be the reason for the sickling of red cells in sickle cell anemia. This disease had been discovered by Dr. James B. Herrick, a cardiologist, in Chicago in 1910 (7). The concept of sickle cell anemia as a “molecular disease” opened a new chapter in medicine (8). Second, Pauling discovered the differential magnetic susceptibility of oxy- and deoxy-hemoglobin, which is the basis of advanced methods of nuclear magnetic resonance imaging, allowing detection of deoxy-hemoglobin in tissues (9) and recently applied to the study of sickle transgenic mice (10).

The Modern period was inaugurated with the discovery by Max Ferdinand Perutz (1914–2002) (*II*) (**Fig. 5**) that the isomorphic replacement method was applicable to large molecules, and that binding of mercury to the Cys93 did not distort the molecule. This solved the phase problem and aided in the description of the tridimensional structure of the hemoglobin molecule. This effort was stimulated by conversations with Felix Haurowitz and realized with the help of a small grant from the Rockefeller Foundation, obtained through the good offices of Sir Lawrence Bragg, inventor of crystallography.



Fig. 5. Max Ferdinand Perutz

As a young investigator, I met Max Perutz in Cambridge shortly after he had published his milestone work, and true to his modesty and bonhomie, he told me that he was happy to have finally published because doing so guaranteed him lab space at the Cavendish, which he was previously at risk of losing for lack of publications. This publication's followup, on deoxyhemoglobin, allowed us to understand the molecular basis of cooperativity and was recognized with the Nobel Prize, which Perutz shared with Kendrew, who had worked on the less complex problem of the crystallography of myoglobin. After the Nobel Prize, he worked even harder. I saw him in Cambridge in the last few months before his death, and we discussed the need to understand why HbC has a highly increased tendency to crystallize. It was a fruitful exchange. We also talked about his leaving Vienna in 1936 to work with J. D. Bernal in crystallography. At the beginning of the war, Britain interned all immigrants born in enemy countries in mild detention camps, even if they were of Jewish origin. Fortunately, the authorities put him to work on plans to construct gigantic ice surfaces that could serve as airplane landing sites in the North Sea. For this plan they needed a crystallographer's knowledge to help them strengthen the crystallized water, at which Max eventually succeeded through the use of wood pulp, although too late to be useful.

Vernon M. Ingram clearly deserves a place in the Modern period. Also in Cambridge, using a sickle cell anemia patient's blood samples left behind by a colleague, he purified the hemoglobin, and ran a trypsin digestion and a combination of electrophoresis and paper chromatography (to be known as "fingerprinting") on the sample, revealing that the mutation in sickle hemoglobin was limited to a single amino acid change: glutamic acid replaced by valine. This proved a momentous finding and the launching of a technique that was used

widely for decades in the analysis of proteins. Vernon wrote a recollection of this discovery later (12).

Another feature of the Modern period is the highly compatible, yet unlikely pairing of Reinhold and Ruth Benesh, called by many R2B2. Reinhold was a Polish immigrant who went to England to study chemistry, and survived by performing stand-up comedy in English vaudeville theaters. He eventually emigrated to the United States, met Ruth, and formed a powerful scientific team. Preparing for a lecture to students of medicine, he realized that 2,3-DPG existed in the red cell in almost identical quantities as hemoglobin. The next day they mixed 2,3-DPG and hemoglobin and observed a right shift of the oxygen equilibrium curve. A new allosteric effector had been found. This finding had tremendous scientific and medical impact. To date, a PubMed search for 2,3-DPG yields 1793 results. Reinhold also contributed to, among other things, the definition of the contact sites in α -chains that contribute to the stabilization of the sickle polymer.

The Post-Modern period, being contemporary, cannot be judged in the same way as the two previous periods. But important accomplishments need to be recognized in the field of hemoglobins. First, George Stamatoyannopoulos merits special mention. Not only has he and his laboratory contributed enormously to the field and trained a slew of young scientists, but he has become the “cheerleader” of research in hemoglobin molecular biology. His “Switching Meetings,” at first in collaboration with Art Nienhuis and George Dover, have become, with time, a classic “George’s show.” Everybody waits for George’s phone call: “What have you done lately?” Stamatoyannopoulos has also been a constant and successful lobbyist to NIH for more money for globin research and for greater opportunities for young investigators to join the field.

The explosion of molecular biology is one of the most important events characterizing this period. Too many important participants are worth mentioning, so I will limit the list to a few that contributed to the field up to 1990 (recent work is outlined in “late-breaking news”):

A. W. Nienhuis	S. H. Orkin
F. G. Grosveld	Y. W. Kan
T. J. Ley	S. L. Thein
L. I. Zon	J. M. Old (<i>see</i> Chapters 7 and 8)
T. M. Towns (<i>see</i> Chapter 13)	T. M. Ryan (<i>see</i> Chapter 13)
J. B. Ligr�el	A. N. Schechter
G. Felsenfeld	E. J. Benz
D. R. Higgs	J. B. Clegg
S. A. Liebhaber	S. M. Weissman
T. Papayannopoulos	B. G. Forget

H. H. Kazazian	N. J. Proudfoot
R. Krishnamoorthy (<i>see</i> Chapter 12)	D. Labie (<i>see</i> Chapter 12)
A. Bank	J. D. Engel
N. P. Anagnou	C. Driscoll
J. L. Sleighton	W. G. Wood
K. Adachi (<i>see</i> Chapter 14)	R. C. Hardison
S. A. Acharya (<i>see</i> Chapter 11)	
and many others.	

Sir David Weatherall also deserves special mention. He is responsible for major developments in the understanding of thalassemia in the last 40 years, including some all-encompassing and very readable textbooks on the subject (**13**). David Nathan is also a major figure in this field in America, contributing to both the scientific and clinical sides (**14**). Clinical advances can be credited to Sergio Piomelli in the general management of this difficult disease (**14**) and to G. Lucarelli (**16**) for his contribution on bone marrow transplantation of thalassemic patients in Italy and the world.

A major and groundbreaking contribution to sickle cell anemia was the discovery by William Eaton and his group that the polymerization of HbS was a nucleation-driven reaction in its two forms: homogeneous and heterogeneous (**17**). In addition, they discovered that the delay time of polymerization was dependent exclusively on the initial concentration of Hb with the potential of modifying the extent of the phenotype (**18**).

The next important discovery in the field, credited to Robert Hebbel and associates (**19**), was the capacity of young sickle cells to adhere to cultured endothelial cells. This finding was confirmed by Dhananjay K. Kaul *in ex vivo* and *in vivo* microcirculatory beds, and was followed by the demonstration that sickle vasocclusion occurred, not predominately in the capillaries as previously thought, but in the small venules, in which the adhesion of young sickle cells preceded obstruction by rigid sickle cells (**20**).

The structure of the sickle polymer was resolved by a combination of the following discoveries: (1) the crystallography of sickle hemoglobin (**21**), (2) the study of the polymerization tendency of binary mixtures of sickle and other hemoglobin mixtures to define residues in the area of contact of the polymer (**22**), and (3) electron microscopy of the polymer and modeling (**23,24**).

Another surprise was the linkage of the sickle mutation with several haplotypes of polymorphic sites in the globin gene cluster. This effort arose based on early work by Y. W. Kan and Stuart Orkin, which was followed by genetic epidemiological studies in Jamaica (**25**), Africa (**26**), and India (**27,28**). Besides demonstrating the multicentric origin of the sickle mutation, this effort revealed the linkage between severity and certain haplotypes and the

role of -158 Xmn I polymorphism in the expression of HbF (29,30), in addition to their power as instruments in anthropological and gene flow studies.

Alpha thalassemia was, after the ameliorating effect of HbF, the first modifier of sickle cell anemia found and most of the credit for this finding belongs to Steve Embury (31).

The discovery of Locus Control Region (LCR), 5' to the β -like gene cluster, by Dorothy Tuan and Irving London (32) had unexpected consequences. In addition to the involvement of LCR in the development of appropriate expression of the β -like globins, it made possible the high and tissue-specific expression of transgenes in mouse models as well as in vectors containing anti-hemoglobinopathies for gene therapy. Although much progress has been made owing to the efforts of George Stamatoyannopoulos, Marc Groudine, and F. G. Grosfeld, a definitive picture has not yet emerged.

The development of transgenic sickle and thalassemic mice, very useful in the field despite unfriendly NIH committee reviews for many years, is a complicated history with many players, so I refer the interested reader to a recent review (33) and Chapter 13.

The successful clinical trial, beyond any rational expectation, of hydroxyurea as a specific treatment for sickle cell anemia (34) is a great landmark in the history of sickle cell in America because it is the only drug approved by the FDA for the treatment of this disease. Investigations leading to this breakthrough involved Paul Heller, Joe DeSimone, George Stamatoyannopoulos, George Dover, and others. The leader of the clinical trial was Sam Character, after years of frustrating rejections by unsympathetic and misguided reviewers, with the competent help of Martin Steinberg (35).

The pioneering work of Chien Ho (*see* Chapter 15) on NMR of hemoglobin and hemoglobin variants was highly successful and contributed to, among other things, the molecular localization of the Bohr protons.

Other less glamorous but equally important clinical advances can be credited to Helen Ranney, who contributed all of her scientific life to hemoglobin research, with her pioneering work on HbA_{1c} as a noted example. She also contributed by organizing what I believe to be the first hemoglobinopathies-dedicated clinic in America, at Jacobi Hospital, Bronx, NY.

The NIH Natural History initiative, under the leadership of Marilyn Gaston (36), saved many lives by demonstrating the effectiveness of penicillin prophylaxis in decreasing infections and mortality in infants with sickle cell disease. The Herculean effort of Graham Serjeant, who headed an MRC unit in Jamaica dedicated to the care and study of sickle cell anemia patients, must be recognized. He produced considerable and reliable natural history clinical data on sickle cell anemia with much less funding than the NIH effort (37).

The discovery of desferrioxamine and the use of chelation therapy to increase the life expectancy of patients with thalassemia major and thalassemia intermedia is also a major accomplishment. The compound is a natural product extracted from actinomycetes, and was reported to be an iron chelator useful in the treatment of hemochromatosis by P. Imhof of Ciba Geigy at the joint annual meeting of the 1962 Swiss Medical in Lugano. An annotation in *Lancet* (38) concludes that “it is unfortunate that in secondary hemochromatosis, usually the result of repeated transfusions in patients with aplastic anemia and other anemias when repeated blood letting is not possible, the drug is apparently less efficacious than in the idiopathic type.” Fortunately, this prediction did not come to pass, and the drug is now the mainstay of the treatment of severe thalassemia. The quest for a clearly effective oral form seems to be close at hand.

Another aspect of research in hemoglobinopathies is the effort to characterize hemoglobin mutants, useful in many of the studies referred to above. In this realm, three investigators have been particularly successful. The first is Herman Lehmann (39,40), who emigrated to Britain early in life, worked in Cambridge, and spent World War II in the British Army in India, in which his training as a hematologist was welcome. He discovered HbS among the “tribals” of India, and contributed profusely to the works on hemoglobin, particularly in the identification and characterization of Hb mutants. He also predicted the duplication of the α -globin loci. The second great figure in this realm was Titus Huisman (41), who published 661 papers in his life, almost all on hemoglobin. He was a refined analytical biochemist and a highly focused and productive researcher. Finally, the successor in this field today is Henri Wajcman, editor of *Hemoglobin*, who runs a highly efficient reference laboratory in Paris for abnormal hemoglobins that has been enormously useful to all of us. Dr. Wajcman is an expert on unstable hemoglobins.

Finally, in “late-breaking news,” the very recent correction of sickle cell anemia (42) and thalassemia (43) by transplantation of stem cells transduced with a lentivirus construct containing human globin genes in mice transgenic models is an encouraging event, and bodes well for the future of gene therapy in hemoglobinopathies.

The remarkably successful adventures that have characterized research and clinical endeavors in hemoglobinopathies have been the product of the efforts of an army of highly qualified and imaginative investigators and clinicians, interested in diseases that affect not only Europe and North America, but most of the third world.

In conclusion, the last century has been good to hemoglobin. Maybe because hemoglobin is red, which helped in its isolation, maybe because it is abundant, or maybe because, as the third book of the Torah (and the Old Testa-

ment) says, “the soul of the flesh is the blood,” hemoglobin has been an active participant in the development of biochemistry, protein chemistry, molecular biology, human genetics, and molecular medicine. It is also apparent that behind it all there was a real network of investigators, sometimes interacting competitively, some times cooperatively, but always in contact. The network has indeed produced a cascade of findings and valuable and unforgettable human interactions. Maybe the lure of this unique and beautiful molecule attracted brilliant, eccentric, imaginative, and one-of-a-kind investigators who blazed a brilliant trail of successes.

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References

1. Brunori, M. (1999) Hemoglobin is an honorary enzyme. *Trends Biochem. Sci.* **24**, 158–161.
2. Gibson, Q. H. (1973) Francis John Worsely Roughton, 1899–1972. *Biogr. Mem. Fellows R Soc.* **19**, 563–582.
3. Wyman, J. and Allen, D. (1958) The problem of the heme interactions in hemoglobin and the nature of the Bohr effect. *J. Polymer Sci.* **7**, 499–518.
4. Edsall, J. T. (1995) Jeffries Wyman, (1901–95) *Nature* **378**, 556.
5. Nagel, R. L. and Gibson, Q. H. (1966) Kinetics of the reaction of carbon monoxide with the hemoglobin-haptoglobin complex. *J. Mol. Biol.* **22**, 249–255.
6. Nagel, R. L., Rothman, M. C., Bradley, T. B., Jr., and Ranney, H. M. (1965) Comparative haptoglobin binding properties of oxyhemoglobin and deoxyhemoglobin. *J. Biol. Chem.* **240**, 4543–2545.
7. Herrick, J. B. (1910) Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. *Arch. Intern. Med.* **6**, 517–521.
8. Pauling, L. (1977) Magnetic properties and structure of oxyhemoglobin. *Proc. Natl. Acad. Sci. USA* **74**, 2612–2613.
9. Ogawa, S., Lee, T. M., Nayak, A. S., and Glynn, P. (1990) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magn. Reson. Med.* **124**, 68–78.
10. Fabry, M. E., Kennan, R. P., Paszty, C., et al. (1996) Magnetic resonance evidence of hypoxia in a homozygous α -knockout of a transgenic mouse model for sickle cell disease. *J. Clin. Invest.* **98**, 2450–2455.
11. King, A. (2002) Restrospective: structural biology and biochemistry. Max Perutz (1914–2002). *Science* **295**, 2382–2383.
12. Ingram, V. M. (1989) Abnormal human haemoglobins. I. The comparison of normal human and sickle-cell haemoglobins by “fingerprinting.” 1958. *Biochim. Biophys. Acta.* **1000**, 151–157.

13. Weatherall, D. and Clegg, J., eds. (2001) *The Thalassemia Syndromes*, Fourth Edition. Blackwell Science, Boston, MA.
14. Nathan, D. G. (1998) *Genes, Blood and Courage: A Boy Named Immortal Sword*. Harvard University Press, Cambridge, MA.
15. Piomelli, S. (1989) Cooley's Anemia Management: 25 years of progress. *Prog. Clin. Biol. Res.* **309**, 23–26.
16. Lucarelli, G., Andreani, M., and Angelucci, E. (2002) The cure of thalassemia by bone marrow transplantation. *Blood Rev.* **16**, 81–85.
17. Eaton, W. A. and Hofrichter, J. (1990) Sick cell hemoglobin polymerization. *Adv. Protein Chem.* **40**, 63–279.
18. Eaton, W. A., Hofrichter, J., and Ross, P. D. (1976) Editorial: Delay time of gelation: a possible determinant of clinical severity in sickle cell disease. *Blood* **47**, 621–627.
19. Hebbel, R. P., Yamada, O., Moldow, C. F., et al. (1980) Abnormal adherence of sickle erythrocytes to cultured vascular endothelium: possible mechanism for microvascular occlusion in sickle cell disease. *J. Clin. Invest.* **65**, 154–160.
20. Kaul, D. K., Fabry, M. E., and Nagel, R. L. (1989) Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: pathophysiological implications. *Proc. Natl. Acad. Sci. USA* **86**, 3356–3360.
21. Wishner, B. C., Ward, K. B., Lattman, E. E., and Love, W. E. (1975) Crystal structure of sickle-cell deoxyhemoglobin at 5 Å resolution. *J. Mol. Biol.* **98**, 179–194.
22. Nagel, R. L., Johnson, J., Bookchin, R. M., et al. (1980) Beta-chain contact sites in the haemoglobin S polymer. *Nature* **283**, 832–834.
23. Edelstein, S. J. (1981) Structure of the fibers of hemoglobin S. *Tex. Rep. Biol. Med.* **40**, 221–232.
24. Watowich, S. J., Gross, L. J., and Josephs, R. (1989) Intermolecular contacts within sickle hemoglobin fibers. *J. Mol. Biol.* **209**, 821–828.
25. Wainscoat, J. S., Bell, J. I., Thein, S. L., et al. (1983) Multiple origins of the sickle mutation: evidence from beta S globin gene cluster polymorphisms. *Mol. Biol. Med.* **1**, 191–197.
26. Pagnier, J., Mears, J. G., Dunda-Belkhodja, O., et al. (1984) Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa. *Proc. Natl. Acad. Sci. USA* **8**, 1771–1773.
27. Kulozik, A. E., Thein, S. L., Kar, B. C., et al. (1987) Raised Hb F levels in sickle cell disease are caused by a determinant linked to the beta globin gene cluster. *Prog. Clin. Biol. Res.* **251**, 427–439.
28. Labie, D., Srinivas, R., Dunda, O., et al. (1989) Haplotypes in tribal Indians bearing the sickle gene: evidence for the unicentric origin of the beta S mutation and the unicentric origin of the tribal populations of India. *Hum. Biol.* **61**, 479–491.
29. Labie, D., Dunda-Belkhodja, O., Rouabhi, F., et al. (1985) The -158 site 5' to the G gamma gene and G gamma expression. *Blood* **66**, 1463–1465.

30. Gilman, J. G. and Huisman, T. H. (1985) DNA sequence variation associated with elevated fetal G gamma globin production. *Blood* **66**, 783–787.
31. Embury, S. H. (1989) Alpha thalassemia. A modifier of sickle cell disease. *Ann. NY Acad. Sci.* **565**, 213–221.
32. Tuan, D. Y., Solomon, W. B., London, I. M., and Lee, D. P. (1989) An erythroid-specific, developmental-stage-independent enhancer far upstream of the human “beta-like globin” genes. *Proc. Natl. Acad. Sci. USA* **86**, 2554–2558.
33. Nagel, R. L. and Fabry, M. E. (2001) The panoply of animal models for sickle cell anaemia. *Br. J. Haematol.* **112**, 19–25.
34. Charache, S., Terrin, M. L., Moore, R. D., et al. (1995) Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. *N. Engl. J. Med.* **332**, 1317–1322.
35. Steinberg, M. H., Lu, Z. H., Barton, F. B., et al. (1997) Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea. Multicenter Study of Hydroxyurea. *Blood* **89**, 1078–1088.
36. Gaston, M. H., Verterm, J. I., Woods, G., et al. (1986) Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. *N. Engl. J. Med.* **314**, 1593–1599.
37. Serjeant, G. R. (2001) The emerging understanding of sickle cell disease. *Br. J. Haematol.* **112**, 3–18.
38. Annotation (1962) A new treatment for haemochromatosis? *Lancet* **i**, **1172**.
39. Lehmann, H. (1984) Sickle cell anemia 35 years ago: reminiscence of early African studies. *Am. J. Pediatr. Hematol. Oncol.* **6**, 72–76.
40. Lehmann, H. (1984) The gradual understanding of thalassemia. *Prog. Clin. Biol. Res.* **165**, 121–136.
41. Proceedings of the Titus H. J. Huisman Memorial Symposium (2001) Augusta, Georgia, USA. June 9, 2000. *Hemoglobin* **25**, 117–258.
42. Pawliuk, R., Westerman, K. A., Fabry, M. E., et al. (2001) Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science* **294**, 2368–2371.
43. Imren, S., Payen, E., Westerman, K. A., et al. (1992) Permanent and panerythroid correction of murine β -thalassemia by multiple lentivirus integration in hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* **99**, 14380–14385.



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