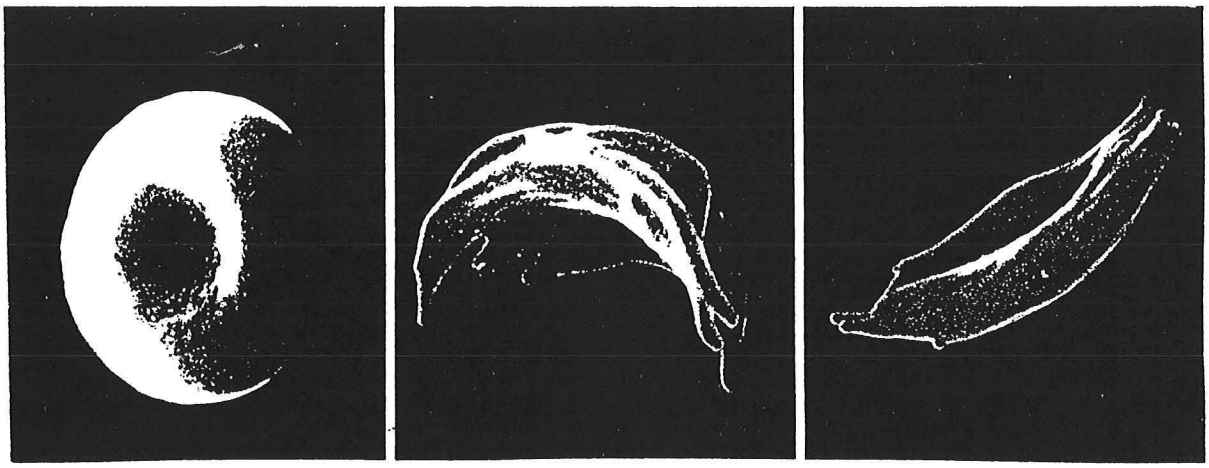
VASCULAR OCCLUSION IN SICKLE CELL DISEASE

PATHO-PHYSIOLOGY AND MANAGEMENT

CYNTHIA J. RUTHERFORD, M.B.,Ch.B



INTERNAL MEDICINE GRAND ROUNDS

UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
DALLAS, TEXAS

FEBRUARY 23 1995

HISTORICAL BACKGROUND

The mutation causing the disease we know today as sickle cell disease arose in Africa about two or three thousand years ago. Although individuals homozygous for the condition have had a very high childhood mortality in Africa, the condition persisted there and increased in frequency because individuals with the sickle trait, the heterozygous state, have a genetic advantage in resistance to malaria, particularly falciparum malaria..(1, 2) In some areas of Africa the frequency of the sickle trait reaches 20-30%.

Long before the characteristic blood cells were observed, Africans recognized sickle cell disease was associated with bone pain, jaundice, fever and early death. (3) Its familial pattern was recognized, often traced through many generations. They knew that in most cases the parents of children with this condition appeared normal and healthy. Different grades of severity of this condition were recognized: "the severe type", probably homozygous sickle cell disease, and the "not so severe type", most likely the double heterozygote sickle C disease. Bones found during an archeological excavation in Nigeria, radio-carbon-dated around 1200 AD, show radiological evidence of hyperostosis and bone infarcts, typical of changes in the bone seen today in sickle cell patients. (4) The Igbo tribe of Nigeria call this condition *ogbanje*, meaning "soon to die" or "born to die". *Ogbanje* children are thought particularly beautiful because of their bossed skulls and high cheek bones, both features of their expanded bone marrow. Because of their physical similarities many thought the *ogbanje* child who died was then reborn in the same family. Even today such children may have part of the little finger cut off early in childhood to make them less attractive to the evil spirits, believed responsible for their premature death. In some tribal languages the name given to this disease are onomatopoeic, reflecting the relentless, agonizing pain of the sickle cell crisis in the bones and joints.

In 1910 the name sickle cell anemia was first given to the condition by James Herrick.(5)The patient reported, Walter Clement Noel was a black dental student from Grenada. The initial description of his blood cells and much of his care came from James Herrick's intern, Ernest Irons, although this was not acknowledged in Herrick's report.(6) Following this description, it was recognized that sickle cell anemia was relatively common, but there was no understanding of its basic etiology, or even its pattern of inheritance for nearly forty years.

When Linus Pauling first learned from William Castle in 1945 that the red cells of patients with sickle cell disease are deformed in the venous circulation, resuming their original shape in the arterial circulation, it occurred to him that sickle cell anemia might be a molecular disease, involving the hemoglobin molecule

This brilliant intuition was tested by Pauling and his post-doctoral student Harvey Itano, using the relatively new technique of electrophoresis on hemoglobin from patients with sickle cell anemia, their parents and normal subjects. They demonstrated the very different mobility of hemoglobin from normal subjects and those with sickle cell disease, and the mixed pattern seen in the parents of patients with sickle cell disease. (7)

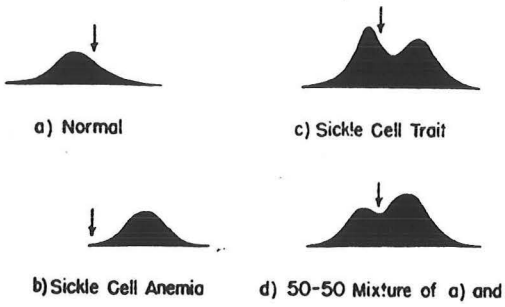


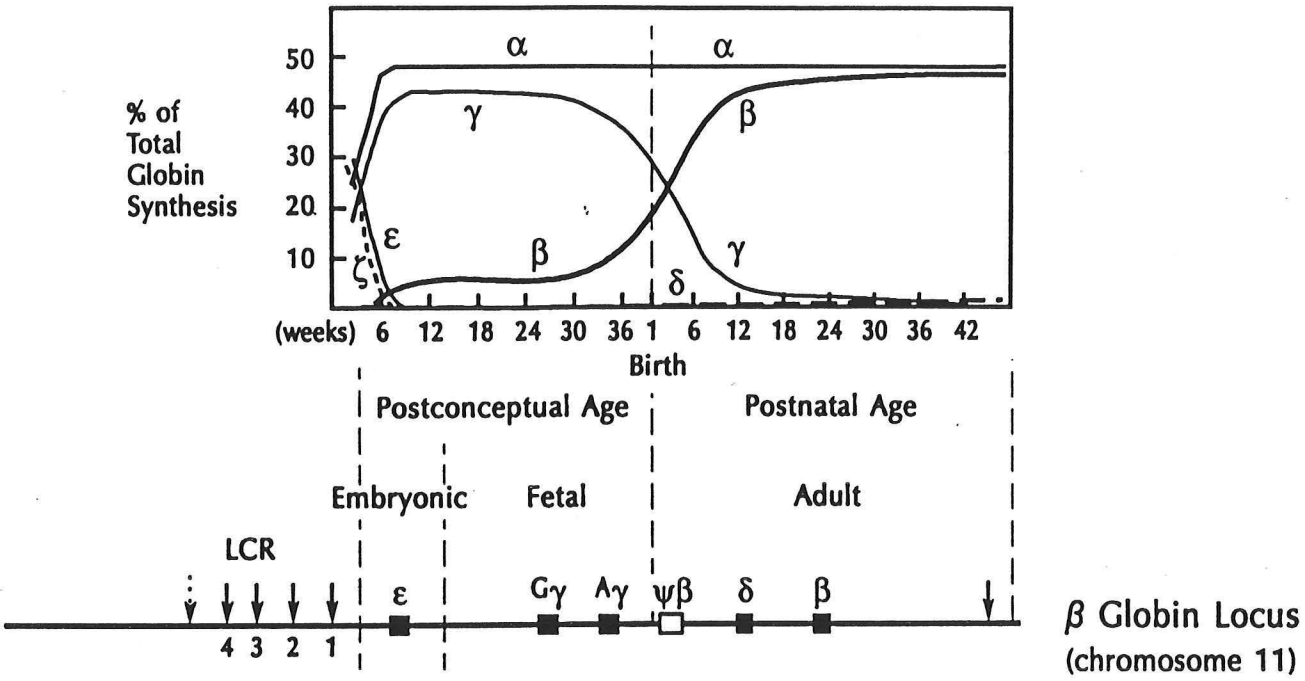
FIG. 3. Longsworth scanning diagrams of carbonmonoxyhemoglobins in phosphate buffer of 0.1 ionic strength and pH 6.90 taken after 20 hours' electrophoresis at a potential gradient of 4.73 volts/cm.

That same year the Mendelian pattern of autosomal recessive inheritance was recognized independently, corroborating their work.(8) Six years later, it was demonstrated by Ingram that these two hemoglobins, despite their very different mobility, differed by only a single amino acid.(9)

MOLECULAR DISEASE

This amino acid substitution is found in the β globin chain of hemoglobin. Hemoglobin is composed of the oxygen-carrying heme moiety and four globin chains. In the adult there are two α and two β chains, forming Hemoglobin A. The α chain synthesis begins very early in embryonic life. The β globin-gene-cluster on chromosome 11 includes the genes for the precursors of β globin which are sequentially switched on and off during embryonic and fetal life. Control of this switching is regulated by the β locus control region or LCR, upstream from the gene cluster. (10, 11) During most of gestation, α chains are paired with γ chains forming fetal hemoglobin (hemoglobin F), which has high oxygen affinity appropriate to the intra-uterine environment. Late in gestation the synthesis of γ chains is progressively switched off, and synthesis of β chains takes over. By the time an infant is six months of age, the fetal hemoglobin level has dropped to the low levels seen in adults, and it is at this age that manifestations of sickle cell disease may begin.

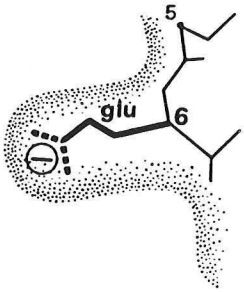
Figure: The β globin gene locus and its products



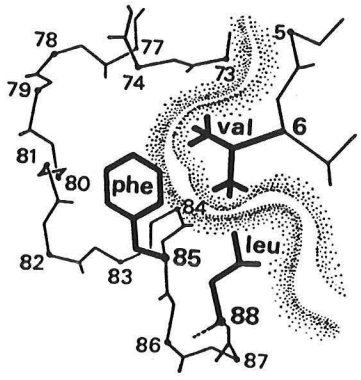
In sickle cell disease the β globin gene differs by a single nucleotide in the sixth residue of the β globin chain, resulting in glutamate acid being replaced with valine The resultant hemoglobin formed by mutant β^S globin is called sickle hemoglobin or hemoglobin S.

β globin codon no	5	6	7
Hemoglobin A	CCT	GAG	GAG
	Pro	Glu	Glu
Hemoglobin S	CCT	GTG	GAG
	Pro	Val	Glu

Normal β^{6Glu} residue



Sickle β^{6Val} residue



The hydrophilic glutamate is a very important amino acid in the complex tertiary structure of the hemoglobin molecule; replacement by hydrophobic valine allows for a hydrophobic contact with a complementary acceptor site on the adjacent β globin chain . This acceptor site is accessible only in the deoxy state due to a slight movement of the interface between the α and β globin chains. The glutamate side chain found in the normal β chain of hemoglobin A is too bulky to occupy this acceptor site. The tight bond between valine and its acceptor site causes the deoxyhemoglobin S to polymerize .

Aggregation of deoxyhemoglobin S into polymers is the primary event in the pathogenesis of sickle cell disease at the molecular level. These polymers distort the usual biconcave disc of the red cell. Transmission electron microscopy demonstrates parallel bundles of long fibers oriented along the sickling axis. Each sickle fiber is a twisted rope-like structure composed of fourteen individual strands, each strand a string of molecular beads of deoxygenated hemoglobin S. (12) The aggregation of polymers of different lengths results in a number of different recognizable sickle cells seen by scanning electron microscopy; shorter aggregations of fibers result in a holly leaf shape.

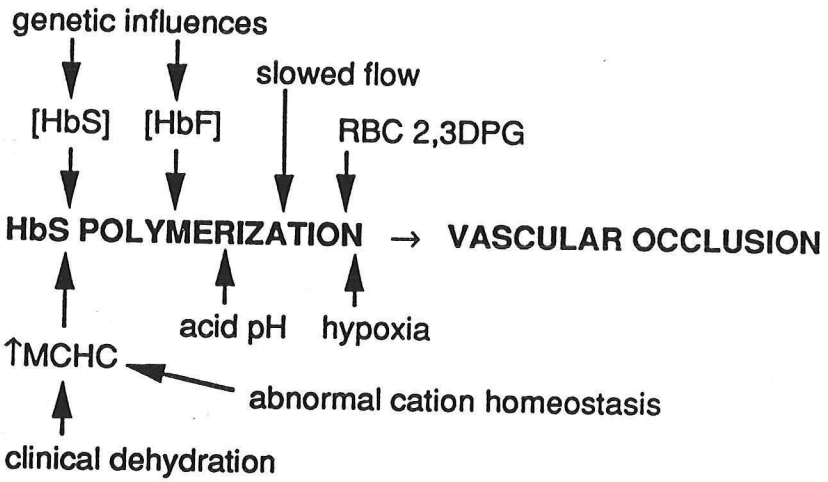
The sickling process occurs primarily during the deoxygenated state , when the alignment of the α and β^S chains changes to enable reaction between the valine and the acceptor site. In sickle cell anemia it is estimated that each circulating red cell undergoes the sickling process approximately four times a minute. Polymerization of hemoglobin S is initiated when the red cell enters the deoxygenated environment of the capillary microcirculation; most red cells are already out of the capillary bed by the time these shape changes occur. Once the red cell is re-oxygenated the hemoglobin S de-polymerizes and the cell resumes its biconcave shape. Repeated cycles of sickling take a toll on the red cell membrane, and eventually the red cell becomes an irreversibly sickled cell. Vascular occlusion in sickle cell disease is initiated in the tiny caliber vessels of the micro-circulation. When these are blocked by sickled red cells a vicious cycle begins, trapping other red cells which become de-oxygenated and undergoing sickling.

What are the events which initiate this micro-vascular occlusion, the key event in the pathophysiology of sickle cell disease? While polymerization of hemoglobin S and formation of a rigid red cell are clearly key components, vascular occlusion is an extremely complicated phenomenon and a number of other factors have been implicated (Table 1).(13-15) There is a tangled web of inter-relationships between these factors which we need to understand so we can devise ways to reduce vascular occlusion.

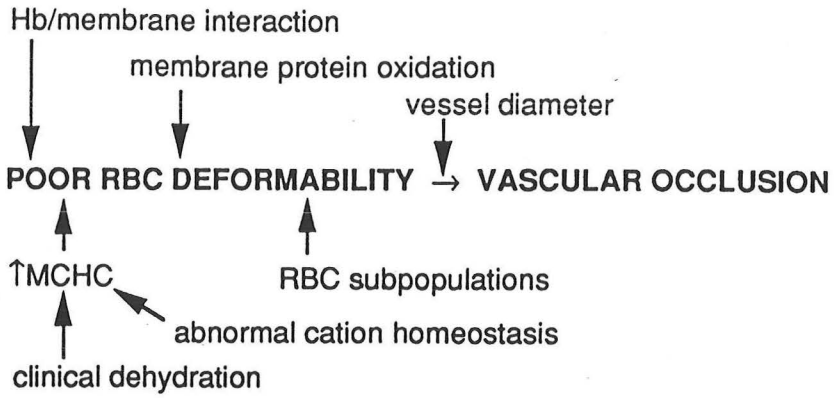
TABLE 1: VASCULAR OCCLUSION IN SICKLE CELL DISEASE: POSSIBLE CONTRIBUTING FACTORS

Hemoglobin S polymer formation
Reduced red cell deformability
Increased blood viscosity
Adherence of sickle cells to endothelium
Endothelial cell activation
Activation of hemostasis
Vascular tone and architecture

Factors predisposing to polymer formation

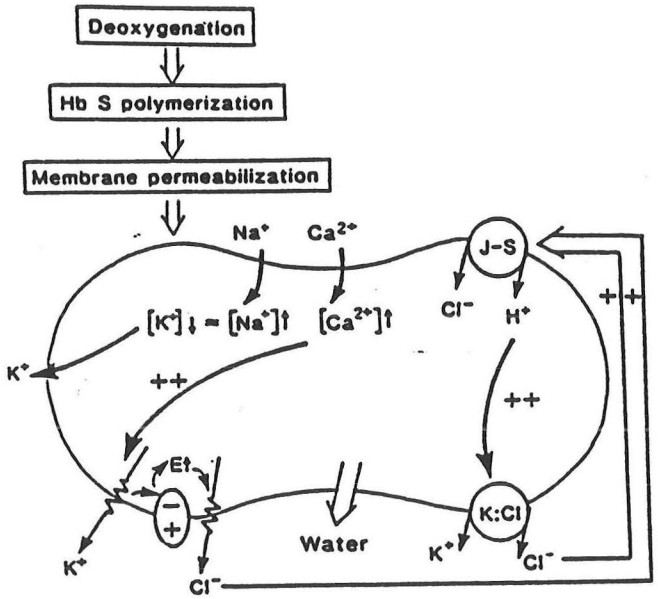


Hypoxia, reduction in pH and slow blood flow are obvious causes of sickle hemoglobin S polymerization, and all promote sickling. Two genetic factors are particularly important because they influence the concentration of hemoglobin S within the red cell which is the key factor for polymerization. The presence of fetal hemoglobin significantly retards sickling, as is seen in infants with sickle cell disease in their first six months and in individuals with sickle cell disease who also carry the gene for hereditary persistence of fetal hemoglobin; some of the milder haplotypes of sickle disease have higher levels of fetal hemoglobin which protect against polymerization. In adults fetal hemoglobin is not evenly distributed through the red cell population, but is restricted to a small subset of cells called F cells, or their younger counterparts, F reticulocytes. *In vitro,* fetal hemoglobin can also be demonstrated to inhibit polymerization, to a degree proportional to its concentration, an effect accentuated by very low oxygen saturation levels.(16) Concurrent α thalassemia also lowers the concentration of hemoglobin S in the red cells, and lowers the MCHC and deters polymerization (17)



The hemoglobin S concentration within the red cell depends on the state of hydration in the red cell. Clinical dehydration may cause an increase in the hemoglobin concentration within the red cell which promotes sickling. However, the major effect is from the abnormal cation homeostasis caused by membrane damage from the repeated cycles of polymerization and depolymerization of sickle hemoglobin. The KCl co-transport channel and the Gardos channel, the calcium activated mechanism whereby water is lost in conjunction with potassium chloride are key players in this homeostasis. Their malfunction can cause dramatic intracellular dehydration and enhanced sickle hemoglobin polymerization.

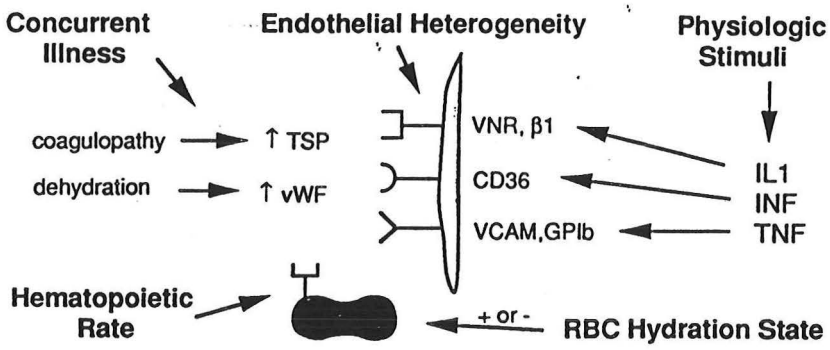
SICKLE RED CELL DEHYDRATION



GARDOS CHANNEL KCl COTRANSPORT

JCI 1987 87: 100

Stractan density gradients of red cells from patients with sickle cell anemia show a very significant population of cells which are very dense due to this dehydration. The poor deformability of such red cells promotes obstruction of the micro-circulation, and the high concentration of hemoglobin S within these dense cells strongly favors polymerization.

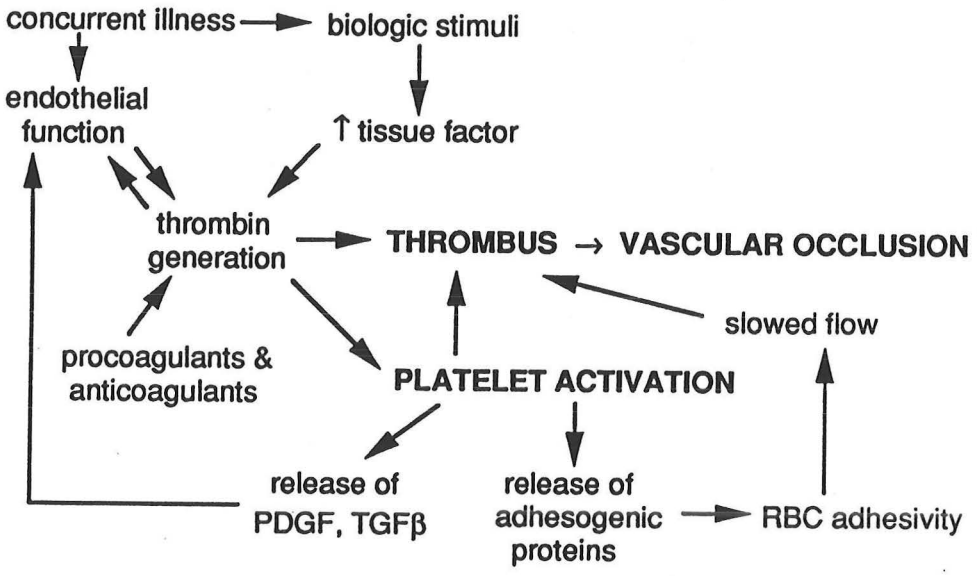


FACTORS INFLUENCING ADHESION OF SICKLE RED CELLS TO ENDOTHELIUM

Abnormalities of the sickle erythrocyte membrane appear to make these cells abnormally adherent both to one another, but particularly to vascular endothelium. The most dense red cells exhibit strong adhesion in regions of low flow, demonstrated by the shear strength required to detach an adherent red cell, because they tend to adhere with multiple contact points. The least dense reticulocyte rich sickle cells are the most adherent in the flow situation. There appear to be multiple mechanisms by which sickle red cells adhere to vascular endothelium: they may bind to a number of receptor sites including von Willebrand receptors, the vitronectin receptor, V-CAM 1, as well as non-receptor-based interaction. Recent experiments by Gee and Platt at the Children's Hospital in Boston have shown that very high levels of adhesion between sickle cells, particularly reticulocytes, and V-CAM 1. (18) A number of the clinical situations associated with vascular occlusive crises are also associated with the type of physiologic stimuli that increased the expression of the implicated receptors. Patients with more severe sickle cell disease and the highest red cell turnover are those with the highest reticulocyte counts and the most potential for vascular adhesion.

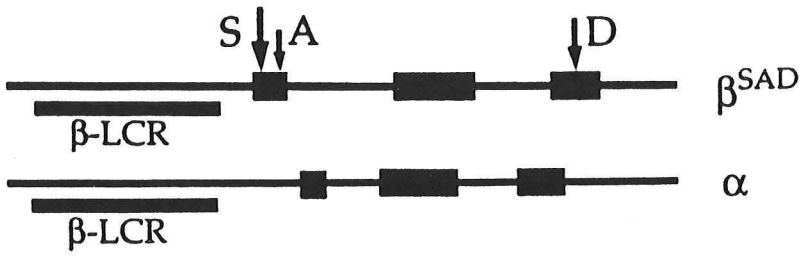
Sickle cell disease has been described as a chronic hypercoagulable state, and for many patients there is evidence of activated hemostasis. (19, 20) Platelet counts are elevated because of hyposplenism; many of these platelets are metabolically-active young platelets. Their increased level of activation is evidenced by the elevated plasma levels of β thromboglobulin.

Some observers have noticed both a drop in the platelet count and reduced platelet survival during acute vascular occlusive episodes. Von Willebrand's factor and factor VIII are also frequently elevated both in steady state disease and during acute episodes. Other supporting evidence for this state of mild, constant activation of coagulation, exacerbated during crises, is suggested by modest reductions in Proteins C and S and factors V and VII, together with elevated fibrin (and fibrinogen) degradation products. Essentially, all three components of Virchow's triad are abnormal in sickle cell disease.



TRANSGENIC MOUSE MODELS FOR SICKLE CELL DISEASE

There is no naturally-occurring animal model for sickle cell disease. However, DNA technology has made it possible to create transgenic animals which produce sickle hemoglobin. Transgenic mice are created by introducing a DNA construct carrying both a human α globin gene and a β globin sickle gene into a fertilized mouse egg; eggs which divide into two cell embryos are selected for re-implantation into a pseudo-pregnant female mouse. Numerous variants of transgenic mice have been made in this way. (21) None exactly mimics the whole spectrum of human sickle cell disease. One of the most promising is the SAD mouse which produces a novel hemoglobin derived from three variants of human sickle hemoglobin genes: normal sickle hemoglobin (β6 glu → val), hemoglobin S-Antilles (β23 val→ile) and hemoglobin D-Punjab(β121 glu→gln). (22)



β^{SAD} and α-globin gene construct used to create SAD mouse

The SAD mice demonstrate many of the features of human sickle cell disease: their hemoglobin polymerizes rapidly and they are very susceptible to hypoxic stress; they have splenomegaly, and renal glomerulopathy; neovascularization of retina and choroid occurs, and males show priapism. Despite these apparently severe manifestations of vascular occlusion, these mice are not anemic. It is interesting to compare the mouse red cell with the human red cell, as the differences may help explain sickle cell disease pathophysiology. The murine red cell membrane has no KCl co-transporter which, in human disease is very important in the generation of dense and irreversibly sickled red cells. Moreover, mouse red cells do not adhere to vascular endothelium. The difficulties in re-creating human sickle cell disease in a mouse model underscore the complexity of the human disease and its varied manifestations.

CLINICAL MANIFESTATIONS OF SICKLE CELL DISEASE

Vaso-occlusive phenomena

Microinfarcts - painful crises
Macro-infarcts - organ damage

Constitutional disturbance

Impaired growth and development
Increased susceptibility to infection

Anemia

Severe chronic hemolysis
Aplastic crises - parvovirus B19

In addition to the manifestations of vascular occlusion, patients with sickle cell dsiease also have a severe chronic hemolytic anemia, where the red cell half life is reduced to 3-13 days, a situation in which an infection which suppresses erythropoiesis, such as parvovirus B19, can cause profound anemia, the so-called aplastic crisis. Both the vascular occlusion and the anemia contribute to the constitutional disturbance, and many sickle cell patients have impaired growth and delayed development. Because of the early vascular occlusive phenomena in the spleen, few patients have adequate splenic function after early childhood leading to an associated susceptibility to infection. Vaso-occlusive phenomena may be acute or chronic. This protocol will deal only with the vascular occlusive phenomena illustrated by the case presented.

VASO-OCCLUSIVE PHENOMENA IN SICKLE CELL DISEASE

ACUTE	CHRONIC
Pain crisis	Aseptic necrosis
Cerebral thrombosis	Retinopathy
Acute chest syndrome	Pulmonary hypertension
	CHF
Splenic sequestration	Splenic atrophy
Hematuria	Hyposthenuria
Priapism	
Hand-foot syndrome	Ankle ulcers

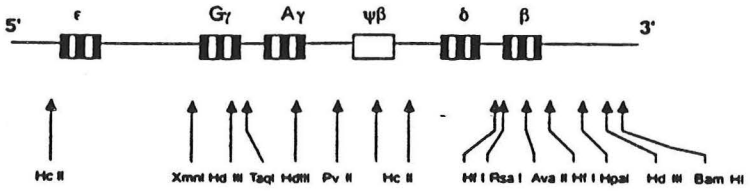
PAIN: The dominating feature for many patients are recurrent attacks of excruciating pain requiring admission for parenteral narcotics. (23, 24) These pains are usually deep-seated bone aching; sometimes pain occurs in the abdomen and may mimic other pathology, sometimes pain involves joints and may be associated with localized swelling. MRI studies have been very helpful in demonstrating the dramatic bone necrosis that is associated with a pain crisis. (25, 26) There are very few other objective signs of these painful crises and for this reason many patients presenting with a sickle pain crisis have been suspected of drug seeking behavior.

Sometimes there will be mild fever, and mildly elevated platelet and white counts; the latter are so non-specific and very much part of the hyposplenic state that they are not helpful. Sophisticated studies usually show initially a rise and then a fall in the number of dense cells, but these measurements are beyond the ability of the normal laboratory to measure; the red cell distribution with (RDW) may decrease, with a loss of the dense cells as the crises progresses. (27). Unfortunately, the changes in the number of irreversibly sickled cells in the blood smear is usually little different statistically from the base line level and is not helpful. The hemoglobin level may fall slightly, but in the setting of the usually generous fluid therapy and associated infection this is difficult to interpret. Similarly, changes in LDH, etc. are not usually dramatic. Sometimes, if the area of bone marrow necrosis is very large, patients may have emboli of necrotic marrow to brain, lungs and kidney, clearly an emergency.

The comprehensive study of sickle cell disease (CSSCD) has looked at the frequency of painful episodes patients with sickle cell disease and has found a marked variation in such episodes. (28) The majority of patients had fewer than one painful crises a year; with age the number of patients having painful crises diminished, possibly associated with death of the patients who are having frequent, severe pain crises, because it is known that shortened survival is linked with an increased frequency of painful crises.(29) Pain frequency was directly proportional to the baseline hematocrit, presumably related to blood viscosity, and inversely proportional to the square of the fetal hemoglobin concentration.

This variation in the frequency of painful crises may be related to the different haplotypes of sickle cell disease. (30)

POLYMORPHISM IN THE β GLOBIN GENE CLUSTER

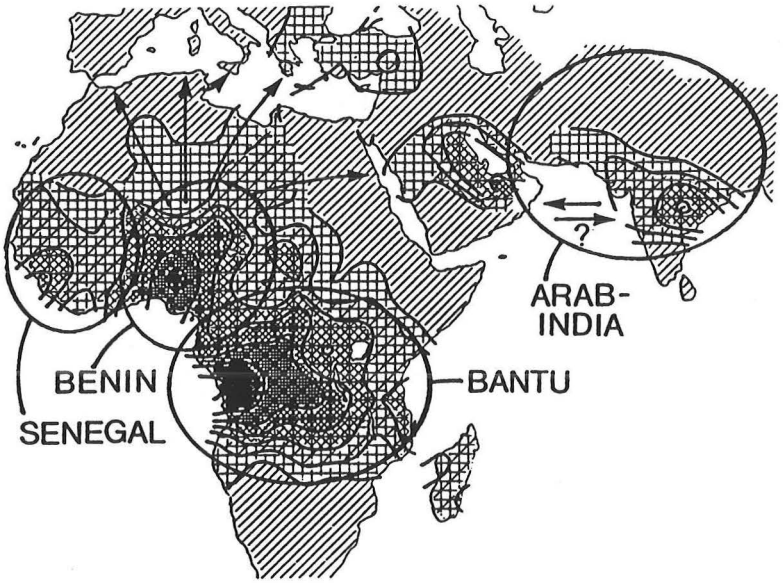


Senegal	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+
Benin	-	-	-	-	-	+	-	+	-	-	+	+	-	-	+
Bantu	-	-	+	+	-	+	-	-	-	+	+	+	+	+	+
Cameroon	-	-	+	+	+	+	-	+	+	-	+	-	+	+	-
Indian	+	+	+	+	-	+	+	+	-	+	+	-	+	+	-

Although all patients with homozygous sickle cell disease have the same β6glu→val mutation in their β globin gene, there are several recognized haplotypes believed to be associated with polymorphism at other sites of the β globin gene cluster. These haplotypes are typical of specific geographical regions and are named for the different areas in which they are believed to have originated. For example, the haplotype Benin in Nigeria: these patients have moderately severe anemia with a hemoglobin between 7-9 grams per deciliter and mean hemoglobin F level of 7.4%; clinically they have severe, painful osteonecrosis. The Benin haplotype is the most common haplotype in the USA.

More severe is the Bantu haplotype in which there is a rapid switch from hemoglobin F in infancy, and subsequent very low hemoglobin F concentrations; these patients have the most severe illness with severe bone and joint pain and growth retardation, and even today such patients often do not survive beyond 20 years of age. In the U.S. this Bantu haplotype is relatively uncommon, comprising about 5% of patients. However, these may be the patients who are frequently seen in emergency rooms.

ORIGINS AND DISPERSION OF SICKLE GENE



Treatment of the pain crises involves treatment of precipitating factors such as infection, dehydration, acidosis, hypoxia and cold, together with administration of intravenous fluids, oxygen, and liberal analgesia. The pain from bone necrosis can be excruciating and usually requires parenteral narcotics, often best administered by a PCA pump. If renal function is normal opioids can be combined with oral or parenteral non-steroidal anti-inflammatory drugs. Many patients with sickle cell disease voice concerns that they are frequently suspected of drug seeking, particularly those who have frequent crises. The incidence of drug addiction in this patient group as a whole in a number of national studies has been from 0 to 10%. Because of concerns about drug-seeking

behavior or causing drug addiction patients are often given ineffective doses of drugs or very short acting medications such as Demerol, which is not a good choice of narcotic for these patients both because of its short duration of action and because of CNS effects from its metabolites which can include convulsions, nervousness, irritability and psychosis.

Last year Dr. George Buchanan and his group at Children's Medical Center in Dallas reported their experience with the use of high dose corticosteroids in children with acute pain crises. They devised this appraoch because the fever and changes in blood components were suggestive of inflammation. (31) They studied 36 children and adolescents with 56 episodes of pain in a randomized double blind study. Patients were randomized to receive 15mg per kilogram methylprednisolone or placebo on admission, with a second dose 24 hours. later. They were given the usual pain relief with IV morphine and transferred over to Tylenol with codeine as pain abated. The severity of the pain crisis as assessed by analgesic use and the duration of hospitalization were compared in the two groups. Both were significantly less in the patients who had received corticosteroids. Unfortunately, the corticosteroid group had a higher incidence of "rebound" pain which makes the overall significance of this study a little difficult to evaluate. A similar study currently underway is using dexamethasone rather than methyl prednisolone, because of its longer duration of action. Dr. Buchanan remains appropriately concerned about the use of corticosteroids in this group of patients who are at higher risk of infection than other patients, and in whom infection and sickle cell vascular occlusive crises may coincide. He cautions use of this therapy outside a tightly-controlled study situation.

Priapism is a frequent complication for males with sickle cell disease, and has been reported from the age of two onwards. (32) Sickle cell disease is responsible for about one quarter of all cases of priapism. The majority of cases reported in the sickle syndromes are in patients homozygous for SS disease. Sickling of red cells occurs within the corporeal sinuses; lack of fibrinogen in blood aspirated suggests that localized intravascular coagulation and subsequent fibrinolysis has occurred. If this condition persists an inflammatory reaction begins resulting in fibrosis. This can lead to impotence, which has an incidence of 25%. In some patients, there is intermittent, so-called "stuttering" priapism which is important to recognize; patients should be warned to present early as 60% of the time, this will proceed to persistent priapism.
Within the first six hours of the onset of priapism, conservative measures including rest, fluids, and oxygen should be tried, but if there is no

improvement, the patient should undergo an exchange transfusion with the aim of lowering the sickle hemoglobin percentage below 30%. If priapism persists, the Urology service should be consulted regarding penile irrigation and direct injection of α-agonists, such as epinephrine. (33) If there is still no improvement with these measures then a shunt procedure will be needed. Radiological studies including MRI may be helpful in determining whether aggressive measures are required at an earlier stage if there is any evidence of necrosis.

TREATMENT OF PRIAPISM

Hours after onset	
< 6	Hydration, analgesia
6-12	Exchange transfusion
12-24	Local aspiration and irrigation with α-adrenergic agonists
> 24	Shunt procedure

Acute chest syndrome is one of the most dreaded complications of sickle cell disease and is the major cause of death in all patients over the age of 5(34-36) It is characterized by the finding of fever, chest pain, infiltrates on chest x-rays, and leucocytosis. The infiltrates on the chest x-rays often begin in the lower lobes, and in about a third of patients, they may be bilateral; 25 to 35% of such patients may have a pleural effusion. Sometimes, this syndrome may appear after anesthesia or other cause for hypoxia. Usually, however, acute chest syndrome appears in a clinical setting where it may be confused with pneumonia, and in some cases it may be precipitated by pneumonia. The primary pathological event appears to be micro-vascular occlusion within the pulmonary micro-vasculature by sickled red cells, but there is often associated thrombosis of medium to small arteries, with or without pulmonary infarction. Clinically, these patients appear acutely ill and may be febrile with cough and pleuritic chest pain - hence the confusion with pneumonia. Hypoxemia is common, and may progress to pulmonary failure. Sometimes this deterioration can be extremely rapid. Management of the acute chest syndrome involves fluids, oxygen; usually antibiotics will be instituted because of suspicion of infection. The patient should be very closely scrutinized with monitored oxygen saturation and respiratory rate. Consideration should be given to exchange transfusion if the condition persists for a prolonged period, if there is deterioration in pulmonary status, or if it is not possible to keep the

$P0_2$ above 70 mm Hg despite intensive oxygen therapy. The goal of the exchange transfusion is to reduce the sickle hemoglobin concentration to 20 or 30% while maintaining a hematocrit of approximately 30%. The differential diagnosis of acute chest syndrome includes pneumonia, ARDS, and fat emboli from extensive bone marrow infarction. A number of patients with acute chest syndrome will subsequently develop some degree of chronic sickle lung disease with parenchymal and vascular fibrosis, which may proceed to pulmonary hypertension. (37)

TREATMENT

Aside from treatment of specific situations associated with vascular occlusion, anemia, infection, etc other treatments may be directed at the underlying pathophysiology. Current approaches under consideration are listed below; only those underlined have had detailed clinical study.

Treatment approaches in sickle cell disease

1. Increasing fetal hemoglobin

   Hydroxyurea ± erythropoietin
   Butyrate

2. Reducing red cell dehydration
   Clotrimazole

3. Antisickling agents

4. Bone marrow transplant

5. Gene therapy

Hydroxyurea

It has long been recognized that patients with sickle cell syndromes who have higher levels of fetal hemoglobin are protected from some of the more adverse consequences of this disease. There are a number of efforts underway attempting to increase synthesis of fetal hemoglobin by reactivating the gene controlling fetal hemoglobin production, an approach

described by Frank Bunn as "reversing ontogeny".(38) Early trials with 5-aza-cytidine in experimental animals and subsequently humans showed increased production of fetal hemoglobin, and it was subsequently realized that a number of other cytotoxic drugs would produce the same phenomenon. (39) The mechanism for this is not clear, but it may involve suppression of normal erythroid progenitors and subsequent recruitment of earlier "stress" erythroid precursors. The latter are involved in the increased red cell production seen after acute blood loss or hemolysis, where newly produced red cells show increased fetal hemoglobin. Because of concerns with difficulty of administration and of late leukemogenic and other risks from many of the agents tried, hydroxyurea was selected as the most appropriate and least risky chemotherapy agent for this approach. Hydroxyurea is administered orally and has not been documented to cause leukemia, even after prolonged use. Its role in sickle cell disease has been under study since the mid 80's. (40-45)

Hydroxyurea is a ribonucleotide reductase inhibitor and inhibits transformation of ribonucleotides to deoxyribonucleotides, an essential step in DNA synthesis. It also appears to inhibit DNA repair. (46) Early studies with hydroxyurea in sickle cell patients showed a significant increase in fetal hemoglobin production in such patients, both in F cells and F reticulocytes. Patients were found to have increased hematocrit, diminished hemolytic parameters, and of course macrocytosis, the latter occurring even before the fetal hemoglobin level rises. Early trials of patients on hydroxyurea were sufficiently promising that in 1992 a multi-center trial was begun to study the role of Hydroxyurea in homozygous sickle cell disease by the National Institutes of Health with the primary aim of assessing the frequency and severity of pain crisis in patients taking this medication. (47) This extremely ambitious and complex trial eventually enrolled 299 adult patients with severe sickle cell disease, defined as three major crises in the preceding year. Hydroxyurea was administered in a randomized double blind manner, so that neither patient nor physician was aware whether the patient was receiving drug or placebo. The complexity arose because risks of bone marrow suppression necessitated two-weekly CBC's which had to be flown to the study headquarters in Baltimore for rapid assessment so the drug could be stopped if bone marrow suppression had occurred. Doses began at 15 mg per kilogram, increasing very slowly to a maximal dose of 35 mg per kilogram, continued for two years. The primary end point was pain and other acute crises, although there are a number of secondary endpoints being assessed, including fetal hemoglobin levels, the effect of macrocytosis.. This trial was scheduled to finish in May 1995, but interval analysis showed such a dramatic difference in the patients receiving the medication that the study terminated early, with an

announcement on January 30 1995 to the effect that the patient receiving the Hydroxyurea showed a 50% reduction in painful crises, in acute chest syndrome, in hospitalizations and in transfusion. These results have appeared in an NIH Clinical Alert, but have not yet been presented at a medical meeting or published in a medical journal.

This study provides a sound basis for using Hydroxyurea in adult patients who have frequent, severe sickle cell crises, and who can be relied upon to comply with the requirements for the frequent blood tests required for dose acceleration and adjustment.. Many remain concerned with prolonged use of a chemotherapeutic agent in a non-malignant disease, and with use of a teratogenic drug in patients in the reproductive age group.

Other agents which increase fetal hemoglobin production

Other pharmacological efforts to increase fetal hemoglobin secretion are on a much smaller scale. In tandem papers in the New England Journal in January 1993, Rodgers reported an further augmentation of fetal hemoglobin in patients when high dose erythropoietin was added to the hydroxyurea therapy (48); in the same issue Perrine reported the use of parenteral butyrate in a short term trial in both patients with sickle cell disease and β thalassemia. (49) The rationale for butyrate use is both the persistently high fetal hemoglobin levels seen in babies born to poorly-controlled diabetic mothers with high serum butyrate levels (50), and the known ability of butyrate analogs to induce hemopoietic cell differentiation.
The three patients with sickle cell anemia on the study showed significant augmentation of fetal hemoglobin, despite the short term nature of the trial. A trial of oral phenylbutyrate has had a similar the effect on fetal hemoglobin production. (51) Studies of other fatty acids and their derivatives are underway.

Clotrimazole

Patients with sickle cell disease have significant abnormalities in the red cell membrane, particularly affecting cation homeostasis There is often profound dehydration of the red cells with high MCHC, and formation of the dense cells seen on the Stractan gradients. A number of substances can inhibit the calcium-activated Gardos channel through which water and KCL are lost from the cell, including the imidazole anti-mycotic drugs. Dr. Carlo Brugnara at the Children's Medical Center in Boston has pioneered the study of Clotrimazole in sickle cell anemia . He showed *in vitro* efficacy of Clotrimazole in inhibiting the Gardos channel and preventing

red cell dehydration (52), demonstrated the same effect in the SAD transgenic mouse (53) and reported a small study of its effect in patients at the recent American Society of Hematology meeting.(54)

Clinical Trial of Clotrimazole (54)

Clotrimazole(CLT): 10 mg/kg/day for 1 week
15 mg/kg/day for 1 week
20 mg/kg/day for 1.5 weeks

	Patient A		Patient B		Patient C	
	Pre CLT	Post CLT	Pre CLT	Post CLT	Pre CLT	Post CLT
Hemoglobin g%	6.7	7.2	7.0	7.4	7.8	8.5
Gardos inhibition (%)	0	52	0	58	0	55
RBC K^+ (mmol/kg Hb)	235	280	240	287	250	305
Dense RBC (%<1.12g/ml)	20.4	0	21.7	7	15.5	2.8

These results showed definite inhibition of the Gardos channel and improvement in intracellular potassium levels, improvement in hematocrit and reduction in the number of dense cells after only a 3-1/2 week trial of this medication. The doses required to produce this effect are very small, less than required for its anti-fungal activity. It is possible that this may prove either a useful adjunct to therapy, or an agent suitable for use by itself.

Bone marrow transplantation

Bone marrow transplantion so far has had a very limited place in the management of patients with sickle cell disease. It is known to be curative in this condition, and early results from European centers predict 86% event-free survival. Most transplants have been performed with HLA-matched sibling marrow after standard conditioning with busulphan and cyclophosphamide.(55, 56) A 22 center collaborative study involving both U.S. and European centers is underway with patient selection criteria delineated in the table. The aim of this study is to undertake transplant soon after complications occur to reduce long term morbidity.

Selection of patients with sickle cell disease for bone marrow transplantation (Collaborative study)

Patients with sickle cell disease, less than 16 years old
One or more of the following complications:

- Stroke or central nervous system hemorrhage
- Impaired neuropsychologic function and abnormal MRI scan
- Recurrent acute chest syndrome
- Stage I to II sickle lung disease
- Sickle nephropathy (GFR 30-50%) predicted
- Bilateral proliferative retinopathy and visual impairment
- Osteonecrosis of multiple joints
- Chronic priapism
- Alloimmunization on chronic transfusion therapy

A preliminary report from this collaborative group was published very recently describing neurologic complications post transplant in 7 of the 21 patients enrolled in the study.(57) Six patients developed seizures of whom five were successfully treated with anticonvulsants; three patients developed intracranial hemorrhage of whom two died. Four of these seven patients had been transplanted because of prior sickle-related stroke, and the three intracranial hemorrhages occurred in patients with a history of stroke. This report suggests that patients with sickle cell anemia are at increased risk of neurologic problems after transplant, and that patients with prior stroke are at risk of intracranial hemorrhage.

Gene Therapy

Gene therapy has not yet been attempted in patients, but is being actively pursued both *in vitro* and using animal models (58) Two approaches are under consideration: incorporation of a β globin DNA with subtle differences in the tertiary structure that interfere with polymer formation.(59) or incorporation of DNA for a normal β globin gene. While this latter approach appears the more promising, there are two stringent requirements which must be met. The normal globin gene needs to be delivered into the pluri-potent stem cell and integrated into its genome so it will persist. The new β globin gene must be expressed at a high level, preferably in the erythropoietic lineage only.(60)

Retroviral vectors have been used to insert normal β globin genes into hemopoietic progenitor cells which carry the β_S gene. This type of vector requires a dividing cell; since the pluri-potent stem cell is usually in a resting phase, retroviral vectors have very low levels of efficiency and

produce very low levels of expression Efforts to improve this efficiency include stimulating the hemopoietic stem cell with growth factors, and devising strategies to incorporate the necessary sequences of the locus control region (LCR) of the β globin gene cluster. This LCR is vitally important for production of high levels of expression of β globin, but unfortunately retroviral vectors cannot tolerate the full DNA sequences of this LCR. Adeno-associated viruses are also being studied because they are believed to able to integrate into the genome of non dividing cells. (61) The future of gene therapy in this group of disorders depends on the ability to isolate and enrich the pluripotent stem cell, and transduce them with a high degree of efficiency.

The last decade has seen a lot of progress in understanding sickle cell anemia. Whereas previously, the process was largely attributed to polymerization of the sickle hemoglobin, it is now realized that vascular occlusion is a much more complicated phenomenon, and our understanding of this process has led to a number of new ways to treat the condition.
If the current problems with gene therapy are solved, this approach holds the exciting prospect of a cure for the disease.

Further reading

The Eighth Day of Creation. by Horace Freeland Judson. Simon and Schuster, New York 1979

The Molecular Basis of Blood Disease, 2nd edition. eds G Stamatoyannopoulos, A.W. Nienhuis. P.W. Majerus, H Varmus. W.B. Saunders 1994

Sickle Cell Disease, 2nd edition. Graham R. Serjeant. Oxford Medical Publications 1992

Sickle Cell Disease, Basic Principles and Clinical Practice. eds S.H. Embury, R.P. Hebbel, N. Mohandas, M.H. Steinberg. Raven Press, New York 1994.

References

1. Cavalli-Sforza LL. The Genetics of Human Populations. Scientific American 1974;231(3):80-89.

2. Friedman MJ, Trager W. The biochemistry of resistance to malaria. Scientific American 1981;244(3):154-155, 158-164.

3. Konotey-Ahulu FID. The sickle cell disease. Clinical manifestations including the sickle crisis. Arch Intern Med 1974;133:611-619.

4. Bohrer SP, Connah GE. Pathology in 700-year-old Nigerian bones. Radiology 1971;98:581-584.

5. Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. Arch Intern Med 1910;6:517-21.

6. Savitt TL, Goldberg MF. Herrick's 1910 case report of sickle cell anemia. The rest of the story. JAMA 1989;261:266-271.

7. Pauling L, Itano HA, Singer SJ, et al. Sickle cell anemia, a molecular disease. Science 1949;110:543-548.

8. Beet EA. The genetics of the sickle cell trait in a Bantu tribe. Ann Eugenics 1949;14:279-284.

9. Ingram VM. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. Nature 1956;178:792-794.

10. Forrester WC, Thompson C, Elder JT, et al. A developmentally stable chromatin structure in the human beta globin gene cluster. Proc Natl Acad Sci USA 1986;83:1359-1363.

11 Tuan D, Solomon W, Li Q, et al. The "beta-like-globin" gene domain in human erythroid cells. Proc Natl Acad Sci USA 1985;82:6384-6388.

12. Eaton WA, Hofrichter J. Hemoglobin S gelation and sickle cell disease. Blood 1987;70:1245-1266.

13. Hebbel RP. Beyond hemoglobin polymerization: the red blood cell membrane and sickle disease pathophysiology. Blood 1991;77:214-237.

14. Francis RB, Johnson CS. Vascular occlusion in sickle cell disease: current concepts and unanswered questions. Blood 1991;77:1405-1414.

15. Serjeant GR, Chalmers RM. Current concerns in haematology. 1. Is the painful crisis of sickle cell disease a"steal" syndrome? J Clin Path 1990;43:789-791.

16. Noguchi CT, Rodgers GP, Schechter AN. Intracellular polymerization: disease severity and therapeutic predictions. Ann NY Acad Sci 1989;565:75-82.

17. Higgs DR, Aldridge BE, Lamb J, et al. The interaction of alpha thalassemia and homozygous sickle cell disease. N Engl J Med 1982;306:1441-1446.

18. Gee BE, Platt OS. Sickle reticulocytes adhere to VCAM-1. Blood 1995;85:268-274.

19. Francis RB. Platelets, coagulation and fibrinolysis in sickle cell disease: their possible role in vascular occlusion. Blood Coagul Fibrinol 1991;2:341-353.

20. Francis RB. Elevated fibrin D-dimer fragments in sickle cell anemia: evidence for coagulation activation during the steady state as well as in painful crisis. Haemostasis 1989;19:105-111.

21. Greaves DR, Fraser P, Vidal MA, et al. A transgenic mouse model of sickle cell disorder. Nature 1990;343:183-183.

22. Trudel M, Paepe ME, Chretien N, et al. Sickle cell disease of transgenic SAD mice. Blood 1994;84:3189-3197.

23. Payne R. Pain management in sickle cell disease. Ann NY Acad Sci 1989;565:189-206.

24. Ballas SK. Treatment of pain in adults with sickle cell disease. Amer J Hematol 1990;34:49-54.

25. Bonnerot V, Montalembert Md, Wioland M, et al. Gadolinium-DOTA enhances MRI of painful osseous crises in children with sickle cell anemia. Pediatric Radiol 1994;24:92-95.

26. Mankad VN, WIlliams JP, Harpen MD, et al. Magnetic resonance imaging of bone marrow in sickle cell disease: clinical, hematologic and pathologic correlations. Blood 1990;75:274-283.

27. Fabry ME, Benjamin L, Lawrence C, et al. An objective sign in painful crisis in sickle cell anemia. the concomitant reduction of high density red cells. Blood 1984;64:559-563.

28. Platt OS, Thorington BD, Brambilla DJ, et al. Pain in sickle cell disease. N Engl J Med 1991;325:11-16.

29. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell diseas. N Engl J Med 1994;330:1639-1644.

30. Powars D. Sickle cell anemia: βS-gene cluster haplotypes as prognostic indicators of vital organ failure. Sem Hematol 1991;28:202-208.

31. Griffin TC, McIntire D, Buchanan GR. High-dose intravenous methylprednisolone therapy for pain in children and adolescents with sickle cell disease. N Engl J Med 1994;330:733-737.

32. Hamre MR, Harmon EP, Kirkpatrick DV, et al. Priapism as a complication of sickle cell disease. J Urology 1991;145:1-5.

33. Molina L, Bejany D, Lynne CM, et al. Diluted epinephrine solution for the treatment of priapism. J Urol 1989;141:1127-1128.

34. Haynes H, Kirkpatrick MB. The acute chest syndrome of sickle cell disease. Amer J Med Sci 1993;305:326-330.

35. Weil JV, Castro O, Malik AB, et al. Pathogenesis of lung disease in sickle hemoglobinopathies. Am Rev Respir Dis 1993;148:249-256.

36. Thomas AN, Pattison C, Serjeant GR. Causes of death in sickle cell disease in Jamaica. Br Med J 1982;285:633-635.

37. Powars D, Weidman JA, Odom-Maryon T, et al. Sickle cell chronic lung disease: prior morbidity and the risk of pulmonary failure. Medicine 1988;67:66-76.

38. Bunn HF. Reversing ontogeny (Editorial). New Engl J Med 1993;328:129-131.

39. Charache S. Hydroxurea as treatment for sickle cell anemia. Hematol Oncol Clin NA 1991;5:571-583.

40. Dover GJ, Charache S. Hydroxyurea induction of fetal hemoglobin synthesis. Seminars Oncology 1992;19(Suppl 9):61-66.

41.Goldberg MA, Brugnara C, Dover GJ, et al. Treatment of sickle cell anemia with hydroxyurea and erythropoietin. N Engl J Med 1990;323:366-372.

42. Goldberg MA, Brugnara C, Dover GJ, al. Hydroxyurea and erythropoietin therapy in sickle cell anemia. Seminars Oncology 1992;19(Suppl 9):74-81.

43. Kaufman RE. Hydroxyurea: Specific therapy for sickle cell anemia. Blood 1992;79:2503--2506.

44. Platt OS, Orkin SH, Dover G, et al. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. J Clin Invest 1984;74:652-656.

45. Rodgers GP, Dover GJ, Noguchi CT, et al. Hematologic responses of patients with sickle cell disease to treatment with hydroxyurea. N Engl J Med 1990;322:1037-1045.

46. Yarbro JW. Mechanism of action of hydroxyurea. Semin Oncol 1992;19(Suppl 9):1-10.

47. TheMulticenter Study of Hydroxyurea. Preventing pain in sickle cell anemia(HbSS) baseline data from patients in a hydroxyurea trial. Blood 1993;82:356a.

48. Rodgers GP, Dover GJ, Uyesaka N, et al. Augmentation by erythropoietin of the fetal hemoglobin response to hydroxyurea in sickle cell disease. N Engl J Med 1993;328:73-80.

49. Perrine SP. A short term trial of butyrate to stimulate fetal-globin-gene expression in the β-globin disorders. N Engl J Med 1993;328:81-86.

50. Perrine SP, Greene MF, Faller DV. Delay in the fetal globin switch in infants of diabetic mothers. N Engl J Med 1985;312:338-338.

51. Dover GJ, Brisilow S, Charache S. Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. Blood 1994;84:339-343.

52. Brugnara C, de Franceschi L, Alper SL. Inhibition of Ca2+-dependent K+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. J Clin Invest 1993;92:520-526.

53. De Franceschi L, Saadane N, Trudel M, et al. Treatment with oral clotrimazole blocks Ca2+-activated K+ transport and reverses erythrocyte dehydration in transgenic SAD mice: a model for therapy of sickle cell disease. J Clin Invest 1994;93:1670-1676.

54. Brugnara C, Gee B, Armsby B, et al. Inhibition of Gardos channel and red cell dehydration by oral administration of clotrimazole in sickle cell disease. Blood 1994;84(Suppl 1):220a.

55. Johnson FL, Mentzer WC, Kalinyak KA, et al. Bone marrow transplantation for sickle cell disease: the United States experience. Am J Pediatr Hematol Oncol 1994;16:22-26.

56. Vermylen C, Cornu G. Bone marow transplantation for sickle cell disease: the European experience. Am J Pediatr Hematol Oncol 1994;16:18-21.

57. Walters MC, Sullivan KM, Bernaudin F, et al. Neurologic complications after allogeneic marrow transplantation for sickle cell anemia. Blood 1995;85:879-884.

58. Kiem HP, Darovsky B, Kalle C, et al. Retrovirus-mediated gene transduction into canine peripheral blood repopulating cells. Blood 1994;83:1467-1473.

59. McCune SL, Reilly MP, Chomo MJ, et al. Recombinant human hemoglobin designed for gene therapy of sickle cell disease. Blood 1994;84(Suppl 1):407a.

60. Stamatoyannopoulos JA. Future prospects for treatment of hemoglobinopathies. West J Med 1992;157:631-636.

61. Luhovy B, MCCune S, Dong JY, al. Stable transfection of AAV vector into normal and sickle adult human hemopoietic stem cells in long term culture. Blood 1994;84(Suppl 1):358a.