

# The paradox of hemoglobin SC disease

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**Abstract** Homozygous HbC gene results only in mild hemolytic anemia. In HbSC disease red cells contain equal levels of HbS and HbC. It is a paradox that HbSC exhibit a moderately severe phenotype in spite of being a mixture of HbS trait and HbC trait, neither of which has significant pathology. Why does the combination of these two Hbs result in a serious disease? The short answer is that HbC enhances, by dehydrating the SC red cell, the pathogenic properties of HbS, resulting in a clinically significant disorder, but somewhat milder than sickle cell anemia (SCA). Nevertheless, retinitis proliferans, osteonecrosis, and acute chest syndrome have equal or higher incidence in HbSC disease compared to SCA.

This pathogenic trick is accomplished by HbC inducing, by mechanisms not fully understood, an increase in the activity of K:Cl cotransport that induces the loss of K<sup>+</sup> and consequently of intracellular water. This event creates a sufficient increase of MCHC, so that the lower levels of HbS found in SC red cells can polymerize rapidly and effectively. This situation offers a unique opportunity: if we could inhibit the effect of HbC on K<sup>+</sup> transport we can cure the disease.

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## INTRODUCTION

Hemoglobin (Hb) C ( $\beta^6\text{Glu6Lys}$ ), is one of the three most prevalent abnormal Hbs of man. The unique pathology of HbC is due to the capacity of HbC to induce erythrocyte dehydration and intracellular crystal formation. The homozygous state of the HbC gene (HbC disease) results only in mild hemolytic anemia. In HbSC disease, where equal concentrations of HbS and HbC coexist, HbC enhances the pathogenic properties of HbS, resulting in a clinically significant disorder. Nevertheless, in most individuals, HbSC disease is clinically milder than sickle cell anemia.

The apparent paradox is that HbSC disease is a compound heterozygous condition, equivalent to a mixture of sickle trait and HbC trait (HbAC), neither of which has significant pathology. Hence, why does the combination of these two Hbs result in a serious disease? To facilitate the understanding of SC disease we need first to review the properties of HbC.

## HbC DISEASE

### The origin and selection of the HbC gene

Most likely, the  $\beta^C$  mutation first occurred among ethnic groups living in Burkina Faso (previously known as Upper Volta).<sup>1</sup> HbC reaches its highest frequency in Central West Africa and its gene frequency decreases concentrically from this epicenter. HbC in Africa is found almost exclusively west of the Niger River and in areas where HbS is also present.

The HbC gene exists at polymorphic frequencies (that is, over 1% gene frequency) in several human populations suggesting that its presence has a selective advantage. HbC is common in malarious areas of West Africa, with an epicenter in Burkina Faso. Modiano et al.,<sup>2</sup> in a large case-control study performed in Burkina Faso on 4348 Mossi subjects, found that HbC is associated with a highly significant 29% reduction in risk of clinical malaria in HbC heterozygotes and a 93% risk reduction in HbC homozygotes. These findings establish that HbC is selected for by *Plasmodium falciparum* malaria and together with the limited pathology of HbAC and HbC disease and the low  $\beta^S$  gene frequency in the geographic epicenter of HbC, also support the hypothesis that, in the long term and in the absence of malaria control, HbC will replace HbS in Central West Africa. The mechanism of the protection appears to involve the interference of HbC with the lysis of parasitized red cells in the late schizont state, impairing the release of merozoites.<sup>3</sup>

### The properties of HbC

In most instances, HbC crystals form in concentrated solutions of purified Hb after incubation with high molarity phosphate buffer.<sup>4,5</sup> The presence of Gln-87 may be responsible for the inhibitory effect of HbF and HbA<sub>2</sub> on HbC crystallization.<sup>6</sup> In contrast, Hb Korle-Bu ( $\beta^{73}\text{Asp6Asn}$ ) accelerates HbC crystallization.<sup>7</sup> The double heterozygote for Hb Korle-Bu and HbC produces a mild microcytic hemolytic anemia and, in vitro, accelerates crystal formation in which precrystal Hb structures convert rapidly into cubic-like crystals as opposed to the typical tetragonal crystal structure of HbC disease, HbSC disease and HbC trait. In vitro crystallization studies led to the conclusion that  $\beta$ -87 and  $\beta$ -73 are contact sites of the oxy crystal. Other similar experiments have led to the conclusion that  $\beta$ -120 and 95 are also contact sites.<sup>8</sup> Crystallographic evidence suggests that HbS, HbC, and HbA have the same molecular structure. However, important areas of the molecule escape precise definition in crystallography (e.g., the N-terminal nonhelical portion of the  $\alpha$  and  $\beta$  chains). Lattice constraints may also favor conformations of molecules in crystals which may not be present in solution.

However, solution experiments<sup>9,10</sup> tell a different story: (1) differences in intrinsic fluorescence indicate that the tryptophane (Trp) microenvironments are more hydrophobic for HbC than HbA; (2) ultraviolet resonance Raman spectroscopy detects alterations in tyrosine (Tyr) hydrogen bonding, in Trp hydrophobicity at the  $\alpha$ 1 $\beta$ 2 interface ( $\beta$ 37) and in the A-helix ( $\alpha$ 14/ $\beta$ 15) of both chains; (3) displacement by inositolhexaphosphate (IHP) of the Hb-bound 8-hydroxy-1,3,6-pyrenetrisulfonate (a fluorescent 2,3-DPG analog) follows the order HbA > HbC; (4) oxygen equilibrium

measurements indicate a differential allosteric effect of IHP in which HbC > HbA; (5) binding of IHP to oxyHbC differs from HbA, indicating perturbations of the oxyHbC central cavity; and (6) a peptide containing 11 residues from the N-terminal portion of band 3, the full cytoplasmic domain of band 3 and 2,3-DPG, accelerate crystallization of HbC by binding to the central cavity of oxyHbC.

More recently,<sup>11</sup> microscopy observations and the nucleation statistics showed that the crystals of HbC nucleate and grow by the attachment of native molecules from the solution and that concurrent amorphous phases, spherulites, and microfibers are *not* the building blocks for the crystal. Thermodynamic analyses of HbC crystallization yielded a very high positive enthalpy, hence HbC crystallization is only possible because of a huge entropy gain, likely stemming from the release of up to 10 water molecules per protein intermolecular contact-hydrophobic interaction. Thus, the higher crystallization propensity of R-state HbC is attributable to increased hydrophobicity resulting from the conformational changes that accompany the HbC  $\beta 6$  mutation.

The mechanism of formation of red cell oxyHbC tetragonal crystals in vivo instead of deoxy crystals is well documented<sup>12</sup> but the crystallization pathway is not totally understood, although new findings are enlightening.<sup>13</sup> Oxy-HbC forms amorphous aggregates that lead to tetragonal crystal formation. Less numerous, are twisted, macro-ribbons that appeared to evolve into crystals. DeoxyHbC, in contrast, forms aggregates and twisted macro-ribbon forms similar to those seen in the oxy state, but resulting in a greater variety of aggregates (polymeric unbranched fibers in radial arrays with dense centers). Unlike the oxy tetragonal crystal, deoxyHbC forms flat, hexagonal crystals. These results imply that: (1) the lys substitution at  $\beta 6$  evokes a crystallization process dependent on ligand state conformation (oxy vs deoxy) and (2) the oxy ligand state is thermodynamically driven to a limited number of aggregation pathways and propensity to tetragonal crystal structure. In contrast, the deoxy form of HbC, energetically equally favors multiple pathways of aggregation, not all of which might culminate in crystal formation.

The above analysis has clinical implications: Why do red cells that contain HbC crystals not induce vasoocclusion? The answer to this riddle is that the crystal forms of oxy and deoxyHbC differ.<sup>12</sup> HbC crystals in the red cells of splenectomized HbC disease patients are in the oxy state and melt readily after deoxygenation.<sup>12</sup> Also, when cells from venous and arterial blood were fixed and counted, there was a significant difference in the mean percent of crystal-containing cells in the arterial circulation vs. the venous circulation ( $1.6 \pm 0.22$  vs.  $1.1 \pm 0.23\%$ ). Hence, the oxyHbC crystals melt before they can do any damage to the microcirculation. In contrast, in sickle cell anemia, the red cell harbor polymerized deoxyHbS as they transverse the microcirculation and obstruct the circulation in small post-capillary venules.<sup>14</sup>

Scanning electron microscopy of cells isolated from density gradients have intracellular HbC crystals.<sup>15</sup> Freeze-frac-ture preparations, followed by electron microscopy, also have intracellular crystals.<sup>3</sup> Circulating crystals can be detected in unperturbed wet preparations from individuals

with HbC disease but they are rare. Together, these observations are consistent with early reports of an increase in intra-erythrocytic crystals in HbC disease following splenectomy.<sup>16,17</sup>

### Red cell properties in HbC disease: morphology and life span

HbC disease red cell morphology is predominately microcytic and hyperchromic, with additional target cells, microspherocytes, and cells with crystalline inclusions.<sup>4</sup> Target cells, a diagnostically useful glass smear artifact, are presumably the consequence of the greater surface to volume ratio of HbC disease cells which is, in turn, the consequence of their reduced water content.

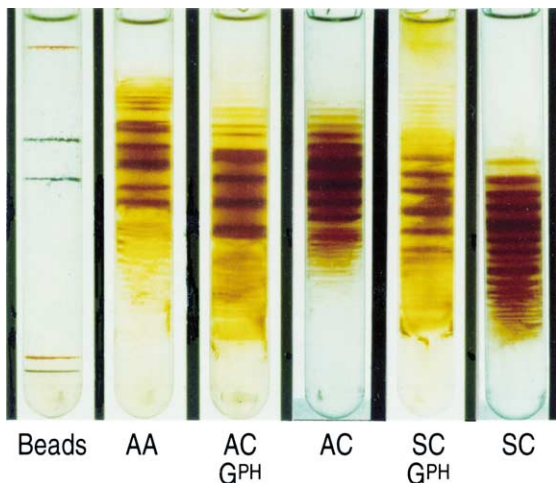
Although red cell life span is shortened to approximately 40 days in HbC disease, this is at least three times as long as the lifespan of cells in sickle cell anemia.<sup>18</sup> This suggests that factors beyond the existence of an elevated population of young cells contributes to the high activity of the K-Cl cotransporter in HbC red cells (see below).

### HbC and red cell density

Erythrocytes that contain HbC have a characteristic high volume-stimulated  $K^+$  efflux. Consequently, intracellular cation and water content of HbC disease cells is strikingly reduced while reduced cation content in HbC trait and HbSC disease cells is less.<sup>19,20</sup> High K-Cl cotransport activity that leads to loss of red cell  $K^+$  and water make the HbC-containing cell dehydrated, increases the intracellular hemoglobin concentration, and makes it more dense than normal. This single feature may be responsible for all of the abnormalities that have been detected in erythrocytes that contain high levels of this Hb.

$K^+$  efflux stimulated by hypotonic conditions (not inhibited by ouabain, butamide, or by charybdotoxin or clotrimazole, inhibitors of  $Ca^{2+}$ -activated  $K^+$  permeability) produces a volume-stimulated decrease in cell water in HbC disease cells.<sup>19,20</sup> The volume-stimulated  $K^+$  efflux occurs at low pH and is  $Cl^-$  dependent and *N*-ethylmaleidamide (NEM) stimulated, establishing that these cells have an active K-Cl cotransport.<sup>21</sup> K-Cl cotransport is present in normal and sickle reticulocytes under oxygenated conditions and its activity decreases with cell aging.

The above events are probably responsible for the well known high MCHC and low intracellular water content characteristics of HbC containing cells.<sup>22,19,20</sup> Red cell density is directly related to the MCHC and red cells in both HbC trait and HbC disease are denser than normal<sup>23</sup> (Fig. 1). Average MCHC in HbC disease, HbSC disease, HbAC trait, and HbA cells were 38, 37, 34, and 33 g/dl, respectively. Red cells of all four of these genotypes have a narrow density distribution so that the youngest and lightest cells differ in concentration is only 3–4 g/dl. In contrast, cells of sickle cell anemia have a very wide density distribution. HbC disease, HbSC disease, and HbC trait reticulocytes are denser than normal reticulocytes. This implies either that these cells are denser than normal when they first enter the circulation or that their density changes within their first 24-h in the circulation when they are still recognizable as reticulocytes.



**Fig. 1 Stractan-Percoll red cell gradients.** The first tube on the left are density beads that allow to detect the gradient of matrix densities in these tubes. The next tube contain normal AA blood. ACG<sup>PH</sup> tube correspond to to a double heterozygote patient for HbC and HbG Philadelphia, an  $\alpha$ -chain mutation. Notice that all cells are move toward to higher density stratas. AC tube has a similar pattern, except thatis has less very dense cells than ACG<sup>PH</sup>. SC tube show uniformly denser cells that AA, AC, and ACG<sup>PH</sup>.

#### Binding of HbC to cell membranes

As is the case for HbS, HbC interacts more strongly with the erythrocyte membrane than HbA.<sup>24</sup> This interaction has been studied by changes in fluorescence intensity of a membrane imbedded probe which is quenched when it is approached by Hb.<sup>25</sup> The cytoplasmic portion of band 3 is the binding site for both HbA and HbC. HbO<sub>ARAB</sub> (with the same charge as HbC) is more strongly bound than HbS and is even more tightly membrane-bound than HbC.<sup>26</sup> These findings suggest that both electrostatic charge and the protein conformation in the vicinity of the charged groups play a role in membrane binding.

All red cells from patients homozygous for HbO<sub>ARAB</sub> are denser than normal red cells, and have a density comparable to that observed for the red cells of HbC disease patients. In HbO<sub>ARAB</sub> heterozygotes, red cell density is strongly influenced by the presence of  $\alpha$ -thalassemia resulting in an average red cell density slightly greater than normal red cells with concomitant - thalassemia. Patients heterozygous for HbO<sub>ARAB</sub>, without  $\alpha$ -thalassemia had a broad distribution of red cell density similar to sickle cell anemia with some cells of normal density but with most cells very dense.<sup>27,28</sup>

Interestingly, compound heterozygotes for HbS and HbO<sub>ARAB</sub> have hemolytic anemia and red cells denser than normal with some as dense or denser that those found in sickle cell anemia.<sup>28</sup> Reticulocytes in patients homozygous for HbO<sub>ARAB</sub> are found in the densest density fraction. Cation transport in patients homozygous for HbO<sub>ARAB</sub> was abnormal, with K-Cl cotransport activity similar to that of HbS-Oman and only somewhat lower than in sickle cell anaemia red cells.<sup>27,28</sup> These data support the conclusion that the red cell pathogenesis of HbO<sub>ARAB</sub> involves the dehydration of red

cells due, at least in part, to the K:Cl cotransport system and that the similarity of the charge and consequences of the presence of both HbC and HbO<sub>ARAB</sub>, strongly suggest that this pathology is the result of a charge-dependent interaction of these Hbs with the red cell membrane and/or its cytoskeleton. Notably, while the mutation in HbC is located at the 6 positions near the entrance to the central cavity, the HbO<sub>ARAB</sub> mutation is at 121, far from the central cavity. This supports a charge-based interaction rather than one that depends strongly on the conformation of the nearby protein.

#### HbSC DISEASE

As we have seen, in HbC a negatively charged  $\beta^6$ -glutamic acid causes a very different pathophysiology than does the hydrophobic  $\beta^6$ -valine present in HbS. The tendency to crystallization, which, along with cell dehydration caused by the HbC induced loss of K<sup>+</sup> and water, is the basis of the pathophysiology of HbC disease, particularly, the hemolytic process. Similar mechanisms contribute to the pathology of HbSC disease with the addition that here, the presence of intra-erythrocytic HbC increases the intracellular hemoglobin concentration by loss of water and K<sup>+</sup>, increasing the tendency of HbS to polymerize.

HbC crystals are most likely to form in cells with low HbF content.<sup>12</sup> When the cells of individuals with HbC disease are density fractionated, cells of highest density had the lowest HbF content. In this dense cell fraction, no F-cells contained HbC crystals. HbF inhibition of HbC crystallization might contribute to the potentially beneficial effects of hydroxyurea in HbSC disease in those individuals who have an increase in HbF in response to treatment with this drug (see below). Vasocclusive episodes are not a feature of HbC disease despite the presence of intra-erythrocytic HbC crystals, for the reasons explained above.<sup>23</sup>

#### The paradoxical pathophysiology of HbSC disease

The paradox of why two mild traits become a life threatening when combined is resolved by the following considerations:

Sickle cell trait and HbSC disease erythrocytes have far lower concentrations of HbS than cells of sickle cell anemia. Yet, the expectation that low HbS concentrations will predict attenuation of polymerization-induced defects is realized only in sickle cell trait. The reason that sickle cell trait and HbSC disease differ in their clinical pathophysiology, has to do with the dehydration of HbSC cells due to an activated K-Cl cotransport.

Cation content of HbSC red cells is intermediate between normal and HbC disease cells. Oxygenated HbSC disease cells exhibit a volume-stimulated potassium efflux similar to that observed in sickle cell anemia and HbC disease.<sup>19,21</sup> This transporter is Cl<sup>-</sup> dependent and NEM (*N*-ethylmaleimide) stimulated.<sup>21</sup> HbSC cells also exhibit a diminished change in cell volume in response to variation of the osmolarity of the suspending medium which is likely to be due to the volume-stimulated potassium efflux.

The consequence of this volume-stimulated K<sup>+</sup> efflux is the dehydration of HbSC cells which by increasing of MCHC will increase HbS polymerization and decrease the delay time

of polymerization, amplifying the effect of the 50% HbS contained in these cells. This effect is the one largely responsible for the observed pathology.

HbSC disease cells share the increased red cell density characteristics of all cells containing HbC (Fig. 1). Reducing the MCHC in HbSC disease to normal levels of 33 g/dl by osmotic swelling results in normalization of many of their polymerization dependent abnormal properties.<sup>23</sup> The beneficial effects of cell rehydration include: increased Hb oxygen affinity (reduced in HbSC disease); reduction of viscosity of deoxygenated erythrocyte suspensions (increased in HbSC disease); fall in the rate of sickling (similar to that in sickle cell anemia); reduction in deoxygenation-induced K<sup>+</sup> efflux.

A less significant event that also contributes to the pathology of HbSC disease is the ratio of HbS to HbC. In HbSC disease, the ratio of HbS to HbC is 50:50, in contrast with the 40:60 ratio of HbS to HbA in sickle cell trait. Several explanations have been proposed for this difference: charge-related differences in  $\alpha\beta$  dimer assembly account for the lower proportion of HbS in sickle cell trait and HbC in HbC trait.<sup>30</sup> Although HbC has one additional positive charge compared to HbS, both compete similarly for  $\alpha$ -globin chains and because of this, the equalization of the HbS to HbC ratio.

Finally, HbF elevation is considerably less than in sickle cell anemia, increasing the expected severity of HbSC disease.

#### HbSC disease and dense cells

A morphological analysis of density-separated HbSC disease cells indicated that they became progressively more aberrant

in shape as their density increased. The densest fraction of the gradient contained cells with the most abnormal shapes which were particularly noticeable for their gross pitting and membrane invaginations.<sup>31</sup> Crystal containing cells are particularly predominant among the highest density fractions.

HbSC disease cells destined to become very dense are likely to produce crystals due to their progressive increase of MCHC (Hb concentration within crystals is about 68 g/dl). Irreversibly sickled cells (ISCs) are rare and associated with the densest cell fractions.<sup>31</sup> In contrast with sickle cell anemia,<sup>32</sup> the highest percent of reticulocytes is found in the dense cell fraction although the very youngest, or stress reticulocytes, are predominant in the least dense fraction.<sup>31</sup> Reticulocytes in HbC disease cells display a similar density pattern in contrast to reticulocytes in normal controls, other hemolytic anemias, and sickle cell anemia, where reticulocytes are the lightest red cells in the blood.

Reticulocytes are almost absent from the densest cell fractions containing crystal laden cells. Hyperdense reticulocytes become less dense if Cl<sup>-</sup> is removed from the media and replaced by NO<sub>3</sub><sup>-</sup>, in concordance with a K:Cl cotransport-mediated event. This interpretation is confirmed by the direct measurements of Canessa et al.<sup>21</sup> where HbSC disease red cells were found to be endowed with an especially active K:Cl co-transport system capable of decreasing volume that was not compensated by Na<sup>+</sup> influx or Na<sup>+</sup>/H<sup>+</sup> exchange.

Scanning electron microscopy<sup>31</sup> (Fig. 2) reveals typical "folded cells," some with a single fold, and resembling - to the gastronomically inclined - pita bread or a taco. These are most likely the cells that Diggs et al. called "fat cells," since

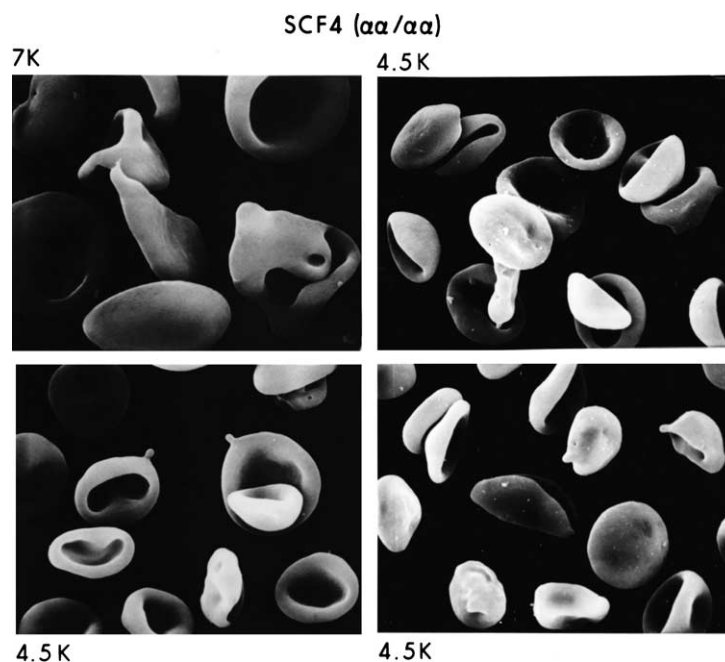


Fig. 2 Scanning microscopy of fractionated SC red cells (having a normal complement of  $\alpha$ -globin genes [ $\alpha\alpha/\alpha\alpha$ ]) corresponding to the most dense red cell fraction (F4) separated by Stractan-Percoll gradients. Notice in the that in the 7 K magnification frame (upper right) multiple folded cells are apparent (one triple dimpled cell), and some of them looking like stomatocytes. In the rest of the 4.5 K frames we observed "pita bread" shapes, stomatocytic forms, "pancake" shaped red cells (that in turn generate "target cells" in the process of making a smear on glass slides).

in Wright stained smears they appear as wide bi-pointed cells.<sup>33</sup> Other misshapen cells had triconcave shapes, that is, triangular cells with three dimples, very much as those seen in acute alcoholism, that Bessis termed "knizocytes". In stomatocytosis there are also triple dimpled cells but they tend to be normal or small diameter. A remarkable shape was the "triple-folded cells" that appeared as two pita breads stuck together (Fig. 2). These bizarre shapes are the product of an increased surface to volume ratio, which provides an excess of surface for the cytosol volume. Excessive surface is resolved largely by membrane folding accounting for the multiplicity of fascinating erythrocyte forms.

Not all cells in HbSC disease contain crystals, in fact, only a small minority do. An enlarged, infarcted, and perhaps abnormally functioning spleen might fail intermittently in its "pitting" function allowing HbC crystals and aggregates to remain in the cell. HbS accelerates the crystallization of HbC in vivo as has been demonstrated in vitro.<sup>34</sup>

#### $\alpha$ -Thalassemia and HbSC disease

As in sickle cell anemia, when  $\alpha$ -thalassemia coexists with HbSC disease fewer dense cells are present.<sup>31,35,36</sup> Also, during the course of painful crisis in HbSC disease, dense cells decrease just as they do during pain episodes in sickle cell anemia, suggesting that painful crisis may be accompanied by dense cell sequestration.<sup>37,38</sup> Coexistence of  $\alpha$ -thalassemia is associated with a decrease in the size of the dense cell fraction and reduction of the number of dense cells containing crystals.

### CLINICAL FEATURES OF HbSC DISEASE

HbSC disease has an incidence of about 1:833 live births in African-Americans.<sup>39</sup> In some West African regions such as Northern Ghana, Burkina Faso, and Western Nigeria about a quarter of the population may have HbSC disease.<sup>40</sup> All complications that are found in patients with sickle cell anemia have occurred in individuals with HbSC disease. Yet, most - but not all - of these complications are seen less often and appear at a later time in HbSC disease compared with sickle cell anemia. Hemolysis is less intense so that anemia is milder and the complications of hemolysis - aplastic episode and cholelithiasis - less frequent or severe. Osteonecrosis of bone is nearly as common, proliferative sickle retinopathy is much more prevalent in HbSC disease than in sickle cell anemia and the mortality of acute chest syndrome may be increased.<sup>41</sup> Painful episodes occur at about half the frequency as in sickle cell anemia. While longer than in sickle cell anemia, the life span of HbSC disease patients is shortened when compared to control populations. Specific treatments that will prevent the complications of this disease are not yet available.

#### Growth and development

Growth in HbSC disease is delayed, but less than in sickle cell anemia. With access to reasonable nutrition, Tanner stages of adult sexual development are achieved 1-2 years earlier than in children with sickle cell anemia.<sup>42</sup> HbSC disease does not appear to affect height, weight, and bone age.<sup>43</sup>

#### Mortality

A 1989 report indicated that 95% of patients with HbSC disease in the United States survived to age 20.<sup>44</sup> Mortality was greatest in children between ages one and three years and commonly the result of pneumococcal sepsis. Another report, showed an age-specific death rate (per 100 person-years) up to age 30 of less than one in HbSC disease compared to two-to-three for sickle cell anemia. Beyond this age, mortality in all patients increased, still, the rate in HbSC disease was less than half that of sickle cell anemia.<sup>45</sup> In the United States, median survival in HbSC disease was 60 years for men and 68 years for women; 3%, or 27 of 844 individuals, died during a 6 year follow-up.<sup>46</sup> There is little data on risk factors for early death in HbSC disease. Median age of onset for irreversible organ failure - stroke, renal failure and chronic lung disease - that might contribute to early mortality in HbSC disease is 10-35 years later than in sickle cell anemia.<sup>45</sup> Increased mortality of HbSC disease is apparent only after the age of 20. The overall prognosis of HbSC disease is therefore better than that for sickle cell anemia.

#### Hematology and laboratory diagnosis

Incubation of SC disease blood with 3% NaCl for 4 h at 37 °C induces the formation of intracellular HbC crystals (Fig. 3). These crystals are the most striking and distinctive feature in circulating red cells of patients with HbSC disease, especially when they have a normal complement of  $\alpha$ -globin genes. HbC crystals have been noted for decades but have only recently been studied intensively using modern approaches. Crystals are observed in Wright's and vital-dye stained smears and in "wet" preparations from finger-stick blood samples (Fig. 4). When  $\alpha$ -thalassemia is present with HbSC disease, typical crystals are absent in some patients. All HbSC disease patients' red cells exhibit heavily stained conglomerations of Hb that appear marginated with rounded edges in distinction to the straight edged crystals. Such cells have been called "billiard ball cells"<sup>31</sup> (Fig. 4). Both crystals and "billiard ball cells" are found in the densest fraction HbSC disease but not sickle cell anemia cells, and represent Hb aggregation distinct from the polymerization of HbS. Regardless of the  $\alpha$ -globin gene haplotype, the blood of HbSC disease patients has additional abnormally shaped cells that are strikingly apparent upon scanning electron microscopy.

Hb electrophoresis, isoelectric focusing, HPLC, and capillary electrophoresis can all accurately determine the relative levels of HbS and HbC. HbS-O<sub>ARAB</sub> and sickle cell anemia/HbG-Philadelphia can appear like HbSC disease on alkaline electrophoresis. In the first case, the distinction can be made by agar gel electrophoresis, where HbO<sub>ARAB</sub> separates from HbC. In sickle cell anemia/HbG-Philadelphia, the hybrid molecule  $\alpha_2^G\beta_2^S$  migrates like HbS at alkaline pH and only HbS is visualized by citrate agar gel electrophoresis. Both of these disorders have a more severe phenotype than the usual cases of HbSC disease and behave more like typical sickle cell anemia. HbE also migrates like HbC at alkaline pH. HbSE disease is usually a mild disorder with minimal anemia, microcytosis and few symptoms.<sup>47</sup>

Smears in HbSC disease contain many target cells and some HbC crystals, the last particularly among patients with

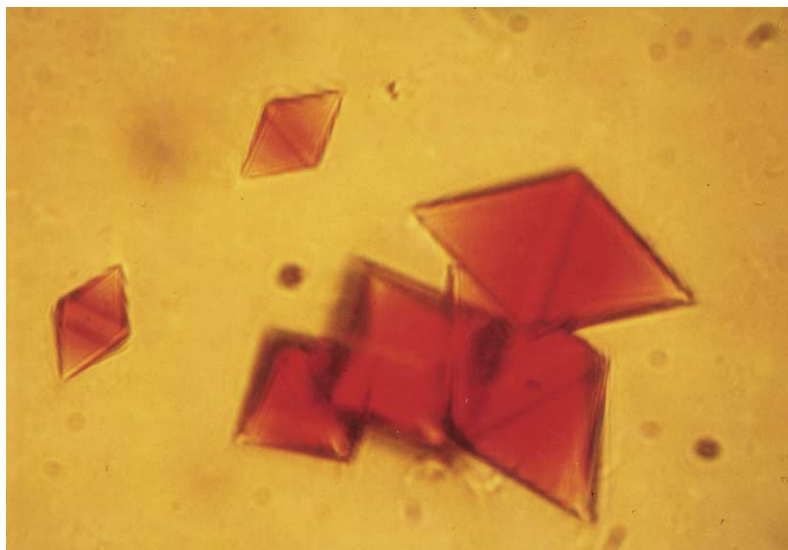


Fig. 3 oxyHbC tetragonal crystals generated in vitro from solution of HbC and high salt media. These are the same crystals observed in vivo in HbC containing red cells.

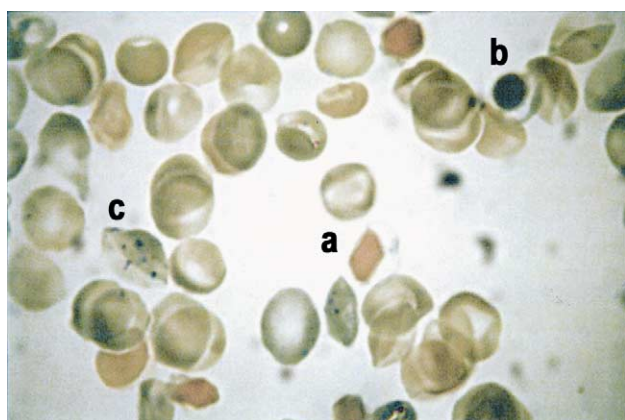


Fig. 4 SC red cells in a smear stain with Wright stain and supra-vital stain: (a) Red cell in which all the hemoglobin has been recruited to a tetragonal crystal, (b) "Billiard ball" cells; (c) A SC reticulocyte in the shape of "pita bread" cell (slide courtesy of Dr. Christine Lawrence).

coexistent of  $\alpha$ -thalassemia.<sup>31</sup> Irreversibly sickled cells are rare. Except in the presence of renal failure, patients with HbSC disease do not usually have symptoms of anemia. The hematocrit in HbSC disease is higher than in sickle cell anemia.<sup>48</sup> Many adult men but fewer adult women have a normal Hct.<sup>48-50</sup> As in sickle cell anemia, high Hct increases blood viscosity and individuals with high Hct may be at greatest risk for complications like proliferative retinopathy and osteonecrosis.<sup>51,52</sup> Leukocyte counts in HbSC disease are normal or only very slightly elevated, perhaps due to persistent splenomegaly and less proliferative activity of the bone marrow. Platelet counts are also lower in HbSC disease than in sickle cell anemia and some individuals have mild thrombocytopenia, often related to an enlarged spleen. The milder

features of HbSC disease might be accounted for by the lower leukocytosis, thrombocytosis, and "stress" reticulocytosis which have been linked to the severity of vasoocclusive disease.

Mild anemia in HbSC disease cannot be entirely accounted for by the rate of red cell destruction, since this does not exceed the potential of normal marrow to replace lost cells. In HbSC disease, the Hct and red cell mass average about 80-85% of normal. As proposed for sickle cell anemia<sup>53</sup> and HbC disease, an increased  $P_{50}$  and enhanced tissue extraction of  $O_2$  might be responsible for the blunted erythropoiesis in HbSC disease.<sup>49</sup>

The oxygen affinity of hemolysates from HbSC disease red cells is normal and red cells had a  $P_{50}$  of 32 mmHg when suspended in isotonic media. When osmolality is reduced to 240 mOsm, normalizing the MCHC, the  $P_{50}$  was restored to a nearly normal 27.5 mmHg.<sup>23</sup>

Hematological features of HbSC disease appear not to be influenced by the  $\beta$ -globin gene haplotype.<sup>54</sup> In African-Americans with HbSC disease,  $\beta^S$  globin gene haplotypes have a similar distribution as in sickle cell anemia.<sup>54,55</sup>

#### HbSC/HbG Philadelphia disease

This genotype has a special phenotype because the hybrid molecule,  $\alpha_2^{G-Philadelphia} \beta_2^C$  increases the rate of crystal nucleation compared to native HbC.<sup>56</sup> HbS enhances crystal nucleation of HbC in a pathogenic relevant manner. Heterozygotes for the  $\beta^S$ ,  $\beta^C$ , and the  $\alpha^{G-Philadelphia}$  genes (HbSC/HbG Philadelphia disease) have abundant circulating intra-erythrocytic crystals and increased numbers of folded red cells with a mild clinical course. Density gradients demonstrate differences in the density pattern when  $\alpha^{G-Philadelphia}$  interacts with sickle trait or SC genotype (Fig. 1) This phenotype seems to be the result of increased crystallization and decreased polymerization caused by the effects of the  $\alpha^{G-Philadelphia}$  globin chain on the  $\beta^C$  and  $\beta^S$  gene

products. Some of the intra-erythrocytic crystals in this syndrome are unusually long and thin, resembling a sugar cane, unlike the typical crystal of HbSC disease.<sup>56</sup> A mild clinical course associated with increased crystallization implies that, in HbSC disease, polymerization of HbS is pathogenically more important than the crystallization induced by  $\beta^C$  chains. HbSC/HbG Philadelphia disease is an example of nonallelic variant hemoglobins interacting to create a unique phenotype.

### Proliferative retinopathy

More common in HbSC disease than sickle cell anemia, the pathology of this entity was described in detail by Welch and Goldberg in 1966.<sup>57</sup> They described and named the distinctive "black sea fan", "black sunburst" and salmon patches that are hallmarks of this disorder and characterized the hemorrhagic, infarctive, proliferative, and resolving lesions of sickle retinopathy. Interacting effects of a higher Hct, increased cell density, and greater blood viscosity in HbSC disease may account for the higher prevalence of retinopathy; it is also possible that the lower HbF levels of HbSC disease play a role. Nevertheless, the exact mechanisms of this pathology is unknown.

Paradoxically, the high prevalence of retinopathy in HbSC disease may be a reflection of the relative benignity of this genotype. In sickle cell anemia, peripheral retinal vessels are occluded early so that further retinal vascular damage and proliferative lesions (neof ormation of leaky retinal vessels) cannot develop. The enhanced circulatory competence of the HbSC disease cell preserves the retinal circulation permitting the later development of proliferative lesions. If this is true, as hydroxyurea treatment makes sickle cell anemia more like HbSC disease, the prevalence of proliferative retinopathy may begin to increase in young sickle cell anemia patients treated with this drug.

Among patients with sickle cell disease attending an ophthalmology clinic, retinopathy was present in a third of patients with HbSC disease compared with three percent of those with sickle cell anemia.<sup>58</sup>

Management of retinopathy is imperfect. Incipient cases have been treated with photo-coagulation to forestall advancing pathology and impaired vision. Some of the earliest comparative trials were inconclusive and questioned the role of photo-coagulation but different techniques of treatment and new technologies now make these results difficult to interpret.<sup>51</sup> There is presently no data that conclusively shows that prophylactic photo-coagulation significantly changes the natural history of retinopathy.<sup>59</sup> Some data suggest that proliferative retinopathy is most prevalent in individuals with higher hemoglobin levels.<sup>60</sup> Phlebotomy, to reduce the hemoglobin concentration to 9–10 g/dl, has also been advocated as a measure to prevent retinopathy or slow its advancement – without evidence of the efficacy of this treatment.

In summary, proliferative retinopathy occurs often in patients with SC disease between the ages of 15 and 30, is progressive, can culminate in visual loss and does not have a definitive treatment that can eliminate its most severe consequences.

### Painful episodes

A rate was about 0.4 painful crises episodes per patient-year has been reported in HbSC patients, less than half the rate in sickle cell anemia.<sup>61</sup> About 60% of HbSC disease patients in Los Angeles reported at least one pain episode.<sup>45</sup>

### Acute chest syndrome

Acute chest syndrome, with its high morbidity in young adults and propensity to evolve to chronic lung disease and appreciable mortality, is seen in about 30% of patients with HbSC disease.<sup>45</sup> Progression to chronic lung disease is only 0.1 that of sickle cell anemia and the median age of onset is almost one decade later.<sup>45,62</sup> Only steady-state leukocyte count was a risk factor for acute chest syndrome in HbSC disease. When compared to sickle cell, there were no differences in length of hospitalization or death rate but sickle cell anemia patients were more likely to present with severe pain.<sup>63</sup>

### The spleen

Splenic function is often preserved in HbSC disease while it is rarely preserved in sickle cell anemia. A positive result of retained splenic function is the reduced incidence of infection by encapsulated bacteria. A negative result is the chance for splenic sequestration crises and splenic infarction to occur in adults. The pathology of the spleen is quite prominent in HbSC disease.

Pocked, or "pitted" red cells are found in increased numbers and are a measure of functional asplenia when splenomegaly is present. Pitted cells increased with age for the sickle cell anemia but not in HbSC disease patients suggesting they have stabilized splenic function<sup>29</sup> for a longer period. However, a discrepancy between pit counting and splenic function tests has been noted in HbC disease.<sup>64</sup> Slightly over one half of all adults with HbSC disease had splenomegaly, 36% were asplenic, and 12% had normal spleens when evaluated by spleen scanning.<sup>50,65</sup>

Six percent of children with HbSC disease had splenic complications that included acute sequestration crisis, painful infarction, and hemorrhage.<sup>66</sup> Similar events can occur in adults with HbSC disease while they are very rare in adult sickle cell anemia.<sup>67</sup> Coexistent hereditary spherocytosis and HbSC disease was deemed responsible for multiple episodes of splenic sequestration crises in one interesting case report.<sup>68</sup>

### Infection

The risk of bacteremia is smaller for HbSC disease patients than for sickle cell anemia patients when correction is made for the unhospitalized population at risk, but is much larger than that for the normal population. Most commonly found in HbSC disease patients are Gram-negative bacteremia, less life-threatening than the pyogenic bacteremia most commonly found in sickle cell anemia.<sup>3</sup> Splenic function is normal, as estimated by "pit" counts in children age four years and less with HbSC disease suggesting that prophylactic penicillin need not be used in this age group.<sup>69</sup>

Some authorities question the use of prophylactic penicillin in HbSC disease.<sup>70,71</sup> A reasonable approach in children who are most susceptible to pneumococcal infection might be to assess splenic function by "pit" counts, the presence of Howell-Jolly bodies, or radionuclide scanning.<sup>72</sup> If splenic function is normal, prophylaxis may be withheld. In sickle cell disease, a large spleen does not always equate with a normal function. Pneumococcal vaccine should be given at age two years and parents instructed to seek immediate medical attention for febrile illnesses.

### Osteonecrosis

Usually painful and often disabling, the incidence of osteonecrosis in HbSC disease is only slightly lower than in sickle cell anemia with an age-adjusted incidence rate of 1.9/100 patient years for the hip joints and 1.7/100 patient years for shoulders.<sup>52,73</sup> Shoulder disease was uncommon in patients less than 25 years old. In one study, osteonecrosis of the femoral heads was almost as prevalent in HbSC disease (8.8%) as in sickle cell anemia (10.2%), results closely echoed by other studies of large patient groups.<sup>45,50,52</sup>

### The kidney

Renal lesions in HbSC and sickle cell anemia are similar. Hematuria is often present and more frequent than in sickle cell trait. In HbSC disease, renal concentrating ability is lost at a time intermediate between the loss in sickle cell anemia and sickle cell trait.<sup>74,75</sup> Renal function deteriorates with age as it does in sickle cell anemia but chronic renal failure is half as common (2.2%) and its median age of onset is 25 years later.<sup>45,48,76</sup> In one study, 73% of 27 patients had papillary necrosis based on calyceal blunting by intravenous pyelography.<sup>65</sup> In this total patient group, renal function was normal and the clinical significance of this observation is unclear.

### Cerebrovascular disease

Two to three percent of HbSC disease patients have stroke,<sup>45</sup> 3–4 times less than in sickle cell anemia. In one large study, 0.8% of individuals with HbSC disease compared to 4% of patients with sickle cell anemia had suffered a stroke.<sup>77</sup> The age-adjusted incidence of stroke in HbSC disease was four times less than in SCA.

### Pregnancy

Perinatal mortality in HbSC disease has been reported to vary from 28% to nil in the absence of transfusions and from 0% to 9% when transfusions were given.<sup>78</sup> Pregnancy-related complications are higher than in normal controls and not dissimilar to those in sickle cell anemia and the rate of Cesarean sections is similar.<sup>79</sup> Although HbSC disease may remain dormant for long periods, stress or pregnancy may result in clinical exacerbation and the pregnant patient may be severely affected.

### Treatment of HbSC disease

No specific therapy for HbSC disease exists. Its paradoxical pathophysiology suggests that the best approach for a clinical "cure" is correcting the erythrocyte dehydration. As dem-

onstrated *in vitro*<sup>23</sup> the beneficial effects of rehydrating HbSC cells inhibit HbS polymer formation. In sickle cell anemia, the change in hydration required to inhibit polymer formation would be much larger because the solubility of HbS alone is about 16 g/dl whereas the solubility (by minimum gelling concentration) of the 50:50 mixture of HbS and HbC is only 2 g/dl less than mixtures of HbS with HbA. Rehydrating HbS cells to decrease polymerization would produce cells that are progressively spheroidal which would decrease their deformability. Nevertheless, modification of intracellular Hb concentration is an attractive therapeutic approach for the treatment of HbSC disease.

This approach has been tested in HbSC cells exposed to an osmolarity capable of their rehydration. At 240 mOsm (normal, 300 mOsm), HbSC cells examined by scanning electron microscopy were normal biconcave disks.<sup>23,80</sup> This represents a proof of principle that the rehydration of HbSC cells leads to a discoidal shape. This shape is indispensable for normal deformation of red cells in the microcirculation.

Several drugs block cation-transport in the red cell and can restore normal cellular cation content and density.<sup>81–83</sup> Clotrimazole, an anti-fungal agent, reduced cellular dehydration *in vitro* in a transgenic mouse model of sickle cell disease and when given to patients with sickle cell anemia.<sup>84–89</sup> Recent studies in sickle cell anemia showed that cell density changes are achievable with well-tolerated doses of this drug.<sup>90</sup> However, the reduction in cell density is modest and less than that observed following hydroxyurea treatment of sickle cell anemia. Derivatives of this agent that are not associated with some of its undesirable effects are now in clinical trials. Magnesium salts also interfere with cation transport and cause cell rehydration, in sickle cell anemia and in sickle transgenic mice.<sup>92</sup> Whether these cellular changes will have clinical utility in HbSC disease is still unknown.

The effects of oral Mg supplementation have been studied *in vivo* in a mouse model for sickle cell disease, the SAD mouse.<sup>91</sup> In this mouse strain, oral Mg supplementation restored red cell Mg and K contents, and reduced K-Cl cotransport activity, MCHC and cell density. Mg pidolate at a dose of 0.6 mEq/kg/day was used as oral Mg supplements in 10 patients with sickle cell anemia.<sup>92,93</sup> Four-weeks of treatment induced an increase in red cell Mg and K content, and a decrease in the activity of K-Cl cotransport. There were no laboratory or clinical signs of hypermagnesemia; mild, transient diarrhea was the only reported side effect. A pilot study, using Mg pidolate for 6-months, confirmed the beneficial effects on red cell dehydration of oral Mg pidolate supplementation and has demonstrated a 58% reduction in the number of painful days. These results represent a promising therapeutic approach for preventing red cell dehydration in sickle cell disease. Two randomized, placebo controlled, cross-over studies are currently evaluating the effects of long-term magnesium supplementation in adult and pediatric patients with sickle cell anemia.

Hydroxyurea can effect changes in the HbSC disease erythrocyte that may be independent of any change in HbF.<sup>94,95</sup> Low dose hydroxyurea is associated with sustained erythrocyte volume increases, a fall in absolute reticulocyte



Table 1 Hematologic findings in 25 patients with HbSC disease treated with hydroxyurea

Patient no.	Age (years)	PCV		MCV		HbF	
		Pre	Post	Pre	Post	Pre	Post
25	8–30	32	34	78	95	1.4	5.6

These data summarize 4 studies of patients of different ages, treated with different doses of drug for different time periods.

counts and numbers of high-intensity staining or “stress” reticulocytes. While Hct increased slightly this is not necessarily a desirable goal for the treatment of HbSC disease since much of the pathology of this disorder may result from increased blood viscosity caused by dense cells. After treatment, density gradients showed a decrease in the number of the most dense cells.<sup>96</sup> Reticulocyte and erythrocyte volume increased, and there was a fall in the percent of red cells and reticulocytes with a CHCM >38 g/dl. Also suggesting a decline in hemolysis was a fall in serum bilirubin. In total, 25 patients with HbSC disease treated with hydroxyurea have been reported (see Table 1). In one study, patients were selected for severe disease. In this uncontrolled trial, before hydroxyurea, patients had 21 admission per year compared with 5 admissions per year after treatment.

The effects of hydroxyurea on renal concentrating ability have been studied in a small group of children with HbSC disease. Treatment with hydroxyurea did not increase the ability to concentrate urine.<sup>97</sup> These data suggest that at the time of treatment with hydroxyurea the countercurrent concentrating mechanism was irreparably damaged. To affect renal medullary function beneficially, hydroxyurea may have to be started in very young children before structural damage occurs.

Transfusions are used in HbSC disease for the same indications as in sickle cell anemia. A recent study has provided much needed information on preoperative transfusion in HbSC disease.<sup>98</sup> Eighteen percent of all patients had some postoperative complication. Postoperative events were compared between the 38% of patients with HbSC disease who received preoperative transfusions versus those not transfused before surgery. Preoperative Hb levels in transfused patients were 12 g/dl with HbA between 35% and 50%. Nine percent of all patients had post operative acute chest syndrome or painful episodes. Nearly 40% of surgery was intra-abdominal and in this group, 35% of patients who were not transfused had an acute chest syndrome or painful episode compared with none who were transfused. Based on these observations and the larger study of preoperative transfusion in sickle cell anemia<sup>99</sup> it appears prudent to use preoperative transfusion in patients with HbSC disease who face procedures with moderate surgical risk.

## CONCLUSIONS

The paradoxical pathophysiology of HbSC disease offers a unique avenue for its treatment and possible clinical “cure”. HbSC disease has a fundamental pathophysiological difference from sickle cell anemia stemming not from the abun-

dance of polymerizing HbS but from the indispensable role of red cell dehydration that brings a 50% HbS to a concentration capable of pathology. The correction of the dehydration of HbSC red cells will correct the polymerization tendency of HbS to the level of sickle trait, leading to the inhibition of the pathology that characterizes this disease.

### Practice points:

- HbC (AC and CC) containing red cells are microcytic (reduced in volume) and have lost water (dehydrated).
- The mechanism of microcytosis and dehydration involve the up-regulation of the K–Cl cotransport, which produces an increase K<sup>+</sup> efflux and with it a loss of intra-erythrocytic water.
- HbSC cells are also microcytic and dehydrated as a consequence of up-regulation of K–Cl cotransport.
- HbSC cells with only 50% of HbS, but because of dehydration (increase in HbS concentration) they are capable of increased HbS polymerization. The consequence a phenotype with significant pathology and after 20 years of age, becomes life threatening.
- While HbSC disease is less severe than sickle cell anemia, some complications are equally severe and in some instances even more severe than in sickle cell anemia: osteonecrosis, retinopathy and acute chest syndrome.
- Nevertheless, if dehydration cause inhibited HbSC diseases would be cured.

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