THE AEROMEDICAL SIGNIFICANCE OF SICKLE-CELL TRAIT: A REVIEW

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This report presents some of the technical background necessary for understanding the aeromedical importance of sickle-cell disease and the sickle-trait carrier, whose erythrocytes contain mixtures of hemoglobin S and normal hemoglobin A. This carrier state (type AS) is not limited to Negroes; it has been found, with lower frequency, in people exhibiting no evidence of African inheritance. Reports of type AS people who died suddenly, exhibiting sickle cells at necropsy, and other reports of sickling crises in these individuals at mild altitudes, have led some authors to conclude that airmen and air passengers who are type AS are at considerable risk. Other reports, particularly those based on the flying experiences of large numbers of pilots with sickle trait, as well as on the results of experimental exposures of type AS people to simulated altitude, indicate that isolated instances of sudden death and altitude intolerance are infrequent in this genotype. The author concludes that a diagnosis of sickle trait, in the absence of a positive history of sickling crises, unusual difficulties in anesthesia, or known contributing factors, is no basis for suspecting an intolerance to moderate altitudes. Individuals with sickle trait who are pilots should be thoroughly trained in the use of oxygen equipment and should avoid any hypoxic experiences, as should all flying personnel.

Stress (Physiology), Hemoglobin, Aviation Personnel, Sickle-Cell Disease, Sickle-Cell Trait, Medical Certification

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I. Introduction.

Travel by air is no longer an unusual luxury to be enjoyed by favored people in a few wealthy nations. Today it is the fastest and often the most economical means of travel. In some parts of the world it is the only practical means of travel, so that the people who now avail themselves of it are not only the healthy, well-nourished Americans and Europeans who have been the subjects of so many aeromedical investigations; today air travelers may be citizens of developing nations or even patients traveling to centers of treatment.

With a greater variety and number of air passengers and crewmembers, we should not be surprised to see a corresponding increase in the variety and number of medical incidents associated with flight. Consider, for example, one of the cases reported by Green, Huntsman, and Serjeant:

A 20-year-old Ghanaian nurse flying an unpressurized aircraft at about 10,000 ft (3,050 m) from Kumasi to Accra developed acute abdominal pain in flight. On arrival she was admitted to hospital with severe pain and board-like rigidity of the whole abdomen. She made an uneventful recovery from what is presumed to be an infarctive incident in the small bowel. Haemoglobin studies later showed she was a sickle-cell trait carrier.

This is one example of many reports associating the sickle-cell gene with crises that can occur under conditions of mild to severe hypoxia. These reports should not be questioned in one respect: Some individuals, whose red blood cells were later shown by screening tests to contain hemoglobin $S$, did indeed suffer some kind of vascular incident under hypoxic conditions. But, as we shall later see, such screening tests—or, for that matter, more sophisticated tests, when evaluated in an unsophisticated manner—are not sufficient to diagnose the genotype of sickle-cell trait.

The report by Green, Huntsman, and Serjeant appeared in December 1972. The following January, interested readers began to suspect that perhaps the risk of altitude to sickle-trait carriers was not so clearly defined as those authors had indicated. Konotey-Ahulu of Accra, Ghana, has had much clinical experience with sickle-cell disease. His letter to the editor of the British Medical Journal drew attention to the report and conclusions of Green et al. as perpetuating “the confusion which has for several decades surrounded the use of the term sickle-cell trait especially in relation to aviation.” Konotey-Ahulu pointed out that a normal haemoglobin (Hb) level in association with a positive screening test does not preclude the coexistence of some other abnormal Hb that could contribute to an altitude intolerance. With reference to the Ghanaian nurse, he warned that “by far the most striking thing about the haemoglobin electrophoresis of sickle cell-$\beta$-thalassaemia is an ‘AS’ pattern with no more fetal haemoglobin than is found in sickle-cell anaemia... . The inexperienced, confronted with such an electrophoretic strip without the control, and without values for $A_s$ and $S$, will almost certainly call it sickle-cell trait.”

Other conflicting reports on hypoxia and sickle trait have appeared in the literature. This paper will review these and other reports.

II. Technical Background.

A primary function of blood is the transport of oxygen ($O_2$) to the other tissues. Critical factors in this transport are (i) the blood’s $O_2$ content, (ii) the rate of mass flow, and (iii) the rate or facility with which the blood acquires $O_2$ in the lungs and releases it to the tissues. An important characteristic of any tissue is its $O_2$ requirement, and this may vary considerably depending on physiological state and tissue type. It is well known, for example, that the central nervous system (CNS) may be irreversibly damaged by low $O_2$ levels that are still adequate for
renal function, the kidneys being second to the CNS in sensitivity to hypoxia. Thus, for any tissue and metabolic state, a certain level of O₂ supply is required; below this level, a condition of insufficiency is said to exist. The O₂ supply to most tissues is normally well above this critical level, as demonstrated by a resting average arteriovenous O₂ difference of 0.041 ml O₂/ml blood, that can increase to more than 0.096 ml O₂/ml blood during periods of increased metabolic demand or decreased blood flow. Part of this ability to extract more or less O₂ from the blood is due to the peculiar shape of the O₂–Hb dissociation curve; Hb releases more O₂ as the pO₂ and pH decrease and as pCO₂ increases.

Variety and Ontogeny of Hb Types. Figure 1 presents a review of this subject. In all Hb types the heme moiety is associated with two pairs of polypeptide chains (denoted here by Greek letters). In normal individuals fetal Hb (HbF) decreases to about 2 percent after birth. The α polypeptides, found in HbF, persist in the normal adult hemoglobins (HbA and HbA₂), but, instead of γ chains, HbA and HbA₂ contains β or δ chains respectively. An inherited failure of α chain synthesis may result in fetal death in the homozygous condition or α-Thalassemia in heterozygous individuals. In Cooley's anemia or β-Thalassemia, an insufficient synthesis of β polypeptide chains results in a severe anemia.
associated with aggregation of α chains in the erythrocyte.

Another interesting condition is the hereditary persistence of fetal hemoglobin (HPFH), which has some influence on the severity of sickle-cell disease and will be considered in detail in another section of this review.

The conditions discussed to this point represent the "quantitative" hemoglobinopathies, those produced by the absence or persistence of entire polypeptide chains. Another group, called "qualitative" hemoglobinopathies, is associated with deletions or substitutions of amino acids in the α or β chain. Stamatoyannopoulos lists 127 of these inherited conditions; some are clinically silent, detectable only by electrophoretic or peptide "finger printing" techniques, while others produce a variety of clinical conditions including methemoglobinemia and polycythemia. Of these 127 qualitative mutations, 42 were associated with α chain substitutions and 85 with β substitutions or deletions. Hemoglobin S (HbS) is included in this latter group along with other hemoglobins that may be of aeromedical significance. For example, Hemoglobinss Olympia and Kansas are associated with abnormal shifts in the O₂-Hb dissociation curve; this is illustrated in Figure 2.

The oxygen affinity of HbS is also abnormal and is an important factor in the sickle crisis, as we will see.

Sickle Hemoglobin. Sickle hemoglobin and others with altered β chain structure are closely related, not only because of structure and inheritance, but also because the severity of and susceptibility to sickle crises in heterozygous individuals are strongly influenced by the type of hemoglobin that is associated with HbS. To fully appreciate this influence, we should first consider the homozygous condition.

Sickle-cell disease (SCD), or sickle-cell anemia, is a condition associated with deformation (sickling) of erythrocytes and consequent vascular insufficiency and pain, especially in the bones and joints. According to Konotey-Ahulu, the condition was recognized in West Africa in ancient times as a disease that "runs through families." Sickle-cell disease is a chronic disease characterized by episodes of painful attacks. Typical signs and symptoms during a crisis are swelling of the liver and spleen (in children), hematuria, painful and swollen extremities, epistaxis, and sudden onset of a gnawing pain in the bones and joints. These attacks are not without permanent effects, which include chronic leg ulcers, maxillary protrusion (from marrow hyperplasia), anemia, and disturbances of growth.

Sickle-cell disease is inherited; at least one HbS gene must be present, but an individual must also inherit an additional abnormal Hb gene before a disease becomes manifest. Phenotypes that qualify in this respect include the hemoglobin mixtures SC, SE, S Thal, and, of course, SS. The term "sickle-cell trait" will be used in this paper to denote combinations of HbS with one of the normal hemoglobins A or A₂ (AS). This usage is consistent with international convention.

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*At least one report has used the symbols "AS" and "SA" to denote two different phenotypes: those of sickle trait and the sickle-thalassemia heterozygote. Such practice is unnecessarily confusing. I will use "AS" to denote the trait phenotype and "S Thal" to denote the result of a heterozygous mixture of HbS with any thalassemic gene.*
In victims of SCD the erythrocytes become deformed and stiffened when the blood pO\textsubscript{2} falls below about 60 mm Hg. Whereas normal erythrocytes are highly elastic and capable of passing through capillaries as small as 3 \(\mu\)m,\textsuperscript{8} the sickled cell is so rigid that it is incapable of passing through holes of much larger diameter. Thus, sickle cells, on becoming deformed, cause tremendous increments in resistance to blood flow.\textsuperscript{9} As blood flow is further reduced, the oxygen tension is lowered further, and more cells become sickled. These events generate a vicious cycle leading to vascular plugging and tissue infarction.

The threshold value of pO\textsubscript{2} at which cells begin to sickle is a function of Hb composition. Figure 3, adapted from a report by Griggs and Harris,\textsuperscript{10} illustrates the differences in susceptibility to hypoxia between red cells containing mixtures of various hemoglobins. Note that HbA confers a marked resistance to hypoxia in cells also containing HbS.

The mechanism by which HbS-containing cells lose their elasticity at low pO\textsubscript{2} is not completely understood. Murayama\textsuperscript{11} has examined the ultrastructural structure of sickled cells and deoxy HbS. His studies reveal the existence in sickled cells of microtubules formed from polymers of HbS. These microtubules are associated in bundles of parallel rods of fibers about 90 nm in diameter; these rods are thought to be responsible for the stiffness of sickled cells.\textsuperscript{b}

**Distribution of the Gene for HbS.** Although it is commonly thought that the sickle gene occurs only in Negroes, it has also been reported in other populations. For example, Chorenis, et al.,\textsuperscript{12} found 12 such individuals in the Greek town of Petromagoula (Orchomenos) near Lake Copais; eight of these gave a positive history of SCD. McGrew\textsuperscript{13} recently reported an incidence of 0.046 percent sickle trait in a population of 57,665 non-Black U.S. Navy recruits. The "African Marker," blood group phenotype oDe, was not found.

It is now generally believed that the presence of HbS in red cells confers some resistance to malaria, especially that caused by *Plasmodium falciparum*.\textsuperscript{14,15} This resistance accounts for the occurrence of a high Hb\textsubscript{S} gene frequency mostly due to a high incidence of the AS genotype in malarious regions. Homozygous SS individuals were fairly short lived, especially in earlier times, and individuals with the AA genotype, being more susceptible to malaria, also died sooner than the heterozygotes. Consequently, the frequency of sickle trait was in excess of 20 percent throughout the low-lying "belt" of Africa before mosquito eradication programs were initiated.

Other reports dealing with the origins and distribution of malaria and sickle disease may be of interest.\textsuperscript{16-18}

### III. Alleged Hazards of Sickle Trait.

The high frequency of the Hb\textsubscript{S} gene in Africa is expected to decrease over the next several generations as a result of mosquito eradication and improvements in housing. Rucknagel,\textsuperscript{19} assuming equal fitness of individuals of genotype AS and AA, estimates that the present gene frequency of about 9 percent in American Negroes will decrease to about 6.3 percent in 10 generations.

Is Rucknagel's assumption of equal fitness of types AS and AA a fair one? Heller\textsuperscript{20} in

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\textsuperscript{b}Other models have been proposed by Magdoff-Fairchild et al., *Nature* 239:217-219 (1972) and by Finch et al., Proc. Natl. Acad. Sci. (US) 70:718-722 (1973).
Chicago studied a population of 3,818 Black male patients, 306 (8 percent) of whom were of the AS genotype. Frequency of sickle trait was essentially the same in all age groups between ages 30–74, an indication that death rates are no higher in individuals with sickle trait than in those of the AA genotype. Petrakis et al. also found no decrease in the frequency of sickle trait in an aging population. In response to a request by the U.S. Department of Defense, the National Academy of Sciences and National Research Council (NAS/NRC) organized the ad hoc Committee on S-hemoglobinopathies to evaluate the importance of sickle trait and other heterozygous states in military activities including aviation. Its report refers to minor renal abnormalities and “isolated” reports of cardiomyopathies associated with alcoholism, as well as some untoward effects of anesthesia, and concludes that although there is some suggestion “that sickle-cell trait is attended by minor morbid consequences and some risks to health, they are of low frequency, and sickle-cell trait is usually benign.”

In contrast to the findings of the NAS/NRC ad hoc committee, there have been a number of reports of sickling phenomena in people with sickle trait and other reports that sickling can be a cause of death in type AS individuals. Some of these reports can be criticized for lack of thorough qualitative and quantitative measurements of the Hb types involved. In some cases of death a finding of sickled cells in the cadaver was interpreted as proof of ante mortem sickling. This interpretation was criticized by Rosenheim, who reminds us “... it should be realized that 8 percent of all Negroes who die suddenly of any cause will have AS hemoglobin and, as McCormick has shown, all with AS hemoglobin will have sickled cells demonstrable at autopsy.” In most necropsies, oxygen tension in the cadaver is well below the levels that produce sickling.

Sudden Unexpected Death. Incidents of sudden unexpected death (SUD) in persons with sickle trait have been the subject of a number of reports. (See Appendix for five cases not previously reported.) Some of these deaths were associated with other suspected contributing factors, such as acute and chronic alcoholism, trauma to the head or chest, and undiagnosed pathological conditions (e.g., pneumonia) not related to hemoglobinopathies. Hypotheses to explain these deaths come readily to mind: any condition that would tend to reduce PO2, generally or in certain tissues, might initiate the cycle of hypoxia, sickling, greater hypoxia, etc. There have been only a few reports of SUD in individuals with sickle trait in which no predisposing conditions could be detected. Jones et al. saw four cases in a population of 4,000 young Negroes during army basic training.

Many of the cases described in the literature have exhibited certain common characteristics: (i) The individual collapses during heavy exercise or an emotional crisis. (ii) He dies soon after collapse, or he appears to recover and then suffers a relapse and dies within 24 h. (iii) One who dies after a number of hours may exhibit before death signs of diffuse intravascular thrombosis, such as a gradual decrease in fibrinogen concentration and an increase in prothrombin time. (iv) Marked vascular congestion and erythrocytic sickling are seen at necropsy. Few will exhibit signs of earlier crises; old infarctions or hemosiderin deposits may be absent.

What is the cause of death in these cases? Given the low incidence of SUD in persons without such known predisposing conditions as trauma, alcohol, and pneumonia, one might assume that some other, unknown contributing factors were present. Certainly, exercise and emotion, though they seem to be initial causes in many cases of SUD, cannot be threats to all those with the trait. Approximately 8 percent of the Black athletes in the U.S. National Football League possess sickle trait. No incidences of SUD are known for these athletes, although it can be safely assumed that they experience greater-than-ordinary physical and emotional challenges. Green, Huntsman, and Serjeant quoted A. E. Romero-Herrera, who stated that

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4 In a letter to the author (April 8, 1975) Dr. David Wieling, Chief Medical Examiner for the Commonwealth of Virginia, writes: “We have occasionally... found persons dead with considerable sickling of their erythrocytes for reasons that are not entirely clear. It would appear that some hypoxic episode has rendered them susceptible to sickling. Some of these antecedent events we believe to be undiagnosed pneumonia, severe alcohol and/or drug intoxication, trauma to the chest, and trauma to the head.”

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no crises occurred in any of the African athletes (some with sickle trait) who participated in the 1968 Olympic games held in Mexico at an altitude of 7,000 ft MSL. The NAS/NRC ad hoc committee was unable to draw any conclusions regarding the risk of SUD in individuals with sickle trait, except that "there must be other, yet unknown contributory factors, not necessarily related to the sickle-cell trait, that make some persons susceptible to collapse or sudden death."

**Altitude Tolerance.** Erythrocytes containing HbS have at least one property not found in other cells; with certain exceptions, only HbS-containing cells become deformed at reduced oxygen tensions. Thus, individuals whose cells possess any amount of HbS might be expected to have less-than-normal altitude tolerance. This reasoning, and reports of sickle crises in flights at various altitudes, have led to the opinion in some medical circles that individuals with sickle trait should not be exposed to altitude (see Reference 30 for a review of the subject). This opinion may seem a prudent and reasonable policy, considering these isolated instances of crises at altitude, but the evidence on which it is based should be reexamined carefully for a number of reasons: (i) Aviation is such a significant means of transport today that individuals denied its use could be seriously affected, both economically and socially. (ii) In many of the developing nations, especially those in Africa, HbS frequency is often in excess of 20 percent. These nations have a great need for air transport; most have national airlines and air freight companies. (iii) In the United States the highest frequency of HbS (about 8 percent) occurs in the Negro segment of the population, which is experiencing significant social and economic changes. It is reasonable to expect an increase in the numbers of Negro air passengers and Negro airmen.

Another reason for the reevaluation of prevailing attitudes toward the altitude tolerance of individuals with sickle trait is the development and recently more common use of techniques for diagnosing the precise genotype of heterozygous individuals and the recognition of other hemoglobin type(s) that may be associated with HbS in the red cell and may significantly affect its response to mild hypoxia (see Figure 3). Many of the reports of sickle crises in flight have failed to indicate the precise Hb composi-
The authors were fully aware that sequestering of deformed cells by the spleen could have concealed the sickling phenomenon and for this reason measured urobinogen for as long as 48 h following exposure; no increased Hb breakdown (as would have been indicated by increments in urobinogen output) was detected. The subject with active anemia, on the other hand, exhibited increased sickling at altitude. His blood, taken at ground level, contained 8 percent sickled cells; this ratio was increased to 9.8 percent at 10,000 ft and 12 percent at 16,000 ft but decreased to 7.2 percent when he began using oxygen.

IV. Contributory and Extenuating Factors in Sickle Crises.

Conflicting reports on the susceptibility of persons with sickle trait to hypoxia, severe exercise, and severe emotional stress were noted earlier in this paper. Although some authors have reported sickle crises in response to these stressors, there are apparently many individuals with sickle trait who can tolerate mild altitudes, compete in demanding athletic contests—even at moderate altitude—and survive the emotional and other challenges of contemporary life.* Even in those with SCD, some variations in the course and severity of the disease have been noted. Although most homozygous (SS) individuals are not likely to survive their fifth decade, some may experience exceptionally mild forms of the disease. Perrine 32 reported a benign form of SCD in natives of Saudi Arabia; Serjeant 33 found mild forms of the disease in Jamaican residents. It may be useful to consider some of the factors that are known to modify the response of HbS-containing cells to hypoxia and to speculate on other factors that might reasonably be suggested as the subjects of further investigations.

Other Hemoglobins. Singer and Singer 24 were probably the first to examine the effects of other types of Hb on the gelling of HbS in the absence of oxygen. They found that mixtures of HbS and HbC, under a continuous flow of CO2, gelled at HbS concentrations lower than those required when HbA was the diluent. Thus, both HbA and HbC may participate in polymerization. In contrast, HbF does not participate. The mildness of SCD in people whose cells contain high titers of HbF—for example, Perrine's 32 subjects—points up the practical value of hereditary persistence of fetal hemoglobin (HPFH) in such cases. The severity of sickling disease in heterozygous individuals is generally consistent with in vitro findings, leading Bertles, 35 for example, to write of “bad” hemoglobins that copolymerize easily with HbS and “good” hemoglobins, such as HbF, that copolymerize weakly or not at all. Milner et al. 36 gives two examples of bad hemoglobin: HbD (Los Angeles) and HbO (Arab). Individuals heterozygous for HbS and these other hemoglobins are afflicted with a disease very similar to that of the HbS homozygote. 35 Those with the SC combination are somewhat less affected, and those with sickle trait are far less troubled by crises. Charache and Conley 37 listed some Hb mixtures in order of increasing severity of disease: AS, SF (HPFH), SC, SD (Punjab), and SS. Possibly the most efficient “good” hemoglobin is HbF, which can exceed 20 percent of the total Hb in heterozygotes for HPFH (genotype AF). 38 More interesting, significant amounts of HbF are found in the red cells of homozygous (SS) persons, and Bertles 35 points out that HbF, which is not uniformly distributed among these cells, contributes to the survival of those containing it. One reason, Bertles professes, for the high titers of HbF in persons with SCD is that cells devoid of HbF irreversibly sickle soon after their release into the circulation; thus, cells containing HbF survive longer and are present in higher numbers at any given time.

Boyer et al. 39 have recently published evidence confirming that HbF is not uniformly distributed among the red cells of normal adults (trace amounts) and those heterozygous for HPFH. These authors did not distinguish between two interpretations of these findings, but both hypotheses are intriguing: either these HbF-containing cells arise from perturbations in β-chain synthesis, producing γ chains—and HbF—in cells of any stem cell lineage, or there exist certain clones of stem cells that, time after time, produce erythrocytes containing HbF. In any case, the production of HbF-containing cells is known to be “turned on” in various conditions including leukemia and pregnancy. Boyer et al.

*Dr. Wieding (loc. cit.) writes: “Why some of these people sickle when others with equivalent sicknesses or injuries don't sickle, I frankly do not know. To a degree, I suppose, our explanation of these cases is a post hoc, ergo propter hoc type of argument.”
suggest that the "selective enrichment of F cells could ameliorate sickle disease," which seems reasonable, provided the number of SS cells containing no HbF could be reduced significantly. Unless this reduction could be accomplished, persons with SCD might still suffer infarctions caused by sickling. To the comments of these authors it should be added that patients with other hemoglobinopathies, notably Cooley's anemia, might also benefit from techniques that could stimulate the production of HbF.

One other compound should be mentioned: methemoglobin (MHB), which is significantly increased in cases of glucose-6-phosphate dehydrogenase deficiency and other conditions, is associated with a milder course of sickling disease when this enzyme deficiency occurs in persons with SCD. Beutler and Mikus\(^9\) induced the formation of MHB in patients with SCD by administering sodium nitrite or p-amino propiophenone. In the blood of patients whose MHB concentrations were at least 20 percent of total Hb, the number of sickled cells occurring at reduced O\(_2\) concentrations was less than in control blood samples, but the O\(_2\)-Hb dissociation curve (ODC) was not materially affected. Sodium nitrite treatments prolonged the survival of erythrocytes \textit{in vivo}, but p-amino propiophenone seemed to decrease survival. Unfortunately, sodium nitrite produces side effects (e.g., headaches) that limit its usefulness. The development of safer techniques for inducing MHB production in man may be a subject worthy of further research.

The Oxygen-Hemoglobin Dissociation Curve. In his review of the problems of O\(_2\) transport in SCD, Milner\(^6\) refers to the shift-to-the-right of the ODC in sickle-cell anemia. He cites the \textit{in vivo} ODC measurements of Bromberg and Jensen,\(^4\) who confirmed earlier \textit{in vitro} studies but in some cases found much lower arterial saturations consistent with not only a right shift, but a slow-rising of the curve in these patients. Milner also refers to the findings of Sproule, Halden and Miller,\(^4\) who found indications of arteriovenous (AV) pulmonary shunts in some patients with SCD. Bromberg and Jensen did not examine their patients for AV shunts, which, if present, could result in a slow-rising of the \textit{in vivo} ODC. Milner concludes that high intracellular levels of organic phosphates—such as 2,3-diphosphoglycerate—are partly responsible for the decreased oxygen affinity of the red cells in SCD (young cells, prevalent in anemia, contain large amounts of these compounds); but the principal cause of the shift-to-the-right, Milner\(^6\) believes, is the presence of large numbers of sickle-damaged cells that possess high concentrations of hemoglobin. This idea is at least consistent with \textit{in vitro} findings, such as those of Rossi-Bernardi \textit{et al.},\(^3\) who demonstrated that HbS obtained from lysed cells may yield an almost normal ODC, far to the left of that obtained with intact cells. As Milner\(^6\) points out, a treatment that could produce a leftward shift in the ODC would benefit the patient with SCD, principally because at higher levels of oxygen saturation the cells are less likely to sickle. Compounds such as urea\(^4\) and cyanate\(^4\) produce leftward shifts in the ODC, and much benefit may be obtained from further study of similar compounds, hopefully derivable from dietary sources. The \(\beta\)-cyanogenic glucosides (nitroligosides, \(\beta\)CG), found in the diets of certain native Africans, have been associated with milder forms of SCD in those who consume these diets in preference to—or in the absence of—more "civilized" foods.\(^4\) The \(\beta\)CG compounds on ingestion yield large amounts of thiocyanates as metabolic products, and some successes in the clinical use of thiocyanates in SCD have been claimed. Thus, the \(\beta\)CG compounds may deserve more intensive study and clinical trial.\(^4\) Except for lastrile (amygdalin), they have not received much attention.

Renal Factors. Hyposthenuria—an inability to fully concentrate urine—is found in at least 80 percent of those with sickle trait and in probably all of those with SCD. This condition is possibly the only clinical sign common to both genotypes; anatomical changes that may explain it have been seen. Status Van Eps and his associates,\(^4\) using a microradiographic technique, demonstrated gross lesions in the renomedullary vessels of people with SCD. In homozygous individuals the vasa recta (efferent arterioles of the juxtaglomerular nephrons), which are responsible for maximum osmolar concentration, were almost completely absent. In the kidneys of four sickle-trait carriers, aged 28, 37, 49, and 82, the vasa recta were reduced in number and bundle architecture was abnormal.

Findings of milder, but similar, pathological changes in people with sickle trait may be sig-
significant, for most authors believe these changes to be associated with the presence of abnormal erythrocytes; but it is not necessary to postulate an unusually severe hypoxemia in the vasa recta of type AS individuals in order to explain their nephropathies. Perilli and Epstein, observing that the sickling of Hbs-containing cells was increased in hypertonic media in vitro, proposed that sickling—and a consequent decrease in medullary blood flow—was most likely to occur in the vasa recta, where the highest osmolarities prevail. The resulting vascular insufficiency could eventually lead to a loss of some of the vasa recta.

Is hypostenuria a threat to those with sickle trait? Considering the high frequency of the condition in persons of this genotype and the rarity of crises in these individuals, the danger from hypostenuria itself must be a minor one; but does the condition sensitize individuals with sickle trait to factors that may cause more serious problems? Consider ethanol. Alcoholism has been associated with sudden unexpected death in persons with sickle trait. Ethanol, a diuretic, may be a contributing factor in cases of SUD, not only because of possible central depressant effects, but possibly also because it contributes toward dehydration. Being unable to efficiently conserve water, hypostenuric individuals may become extremely dehydrated. Even in the absence of the concentrating mechanism of the vasa recta, the kidney retains some minimal ability to concentrate urine, so that the renal blood may possess the high osmotic pressures that lower the sickling threshold. It is, therefore, reasonable to speculate that in a dehydrated individual with sickle trait, the kidney might be an important site for the initiation of a sickle crisis.

Other evidence that osmotic stress may damage erythrocytes is the finding by Alexander and coworkers that in dogs restricted to 250 ml of water per day for 5 weeks, the erythrocyte survival time was 14 percent of normal. In chronic alcoholics, subjected to daily cycles of dehydration-rehydration, a similar damage to erythrocytes might result.

**Drugs and Hormones.** In addition to effects on circulation and respiration and, hence, on the sickling process, certain drugs and hormones may produce direct changes in the red cell and the red cell mass. Valeri presents an excellent review of some of these changes. For example, abnormally high levels of catecholamines (e.g., in pheochromocytoma) may cause reductions in red cell mass, and a number of drugs are known to produce an immunologic hemolytic anemia.

As noted earlier, the intracellular levels of organic phosphates—particularly 2,3-diphosphoglycerate (2,3-DPG)—are at least partly responsible for the shift-to-the-right in the ODC of individuals with sickle-cell disease. The erythrocyte content of 2,3-DPG can be increased by a number of hormones including growth hormone, thyroxine, epinephrine, and androgens; in contrast, parathormone is known to effect a decrease in 2,3-DPG. Böning et al. reported a diurnal variation in the erythrocyte content of 2,3-DPG; peak levels were seen in the afternoon and the lowest levels, between 0100 and 0900.

Changes in erythrocyte deformability have also been induced by drugs and hormones. Prostaglandin E₂ (PGE₂) was reported by Allen and Rasmussen to reduce red-cell deformability (measured by a filtration technique) at PGE₂ concentrations as low as 10⁻¹¹ M; the maximum effect was seen at 10⁻¹⁰ M. Epinephrine and DL-isoproterenol had a similar effect, but higher concentrations (10⁻⁹ and 10⁻⁷ M) were required. The report by Johnson et al. that PGE₂ induces and potentiates sickling in vitro under hypoxic conditions may indicate a role for prostaglandins, and perhaps catecholamines, in the sickling crisis. Clinical trials of aspirin, an inhibitor of PGE₂ synthesis, seem indicated. Propranolol, the β-adrenergic blocking agent, binds to erythrocytes and might protect them from the effects of catecholamines.

**Hemostatic Mechanisms and Plasma Proteins.** An investigation of the effects of aspirin on the frequency and severity of sickling crises seems doubly warranted in light of Rickles and O'Leary's report that platelet consumption is increased during the sickle crisis. This action leads to the release into the circulation of young platelets possessing an increased sensitivity to aggregants, such as adenosine diphosphate, thrombin, and, perhaps, PGE₂. It will be recalled that the sudden unexpected death of people with sickle trait may involve an initial collapse followed by partial recovery, a second collapse, and death in coma with signs of diffuse
intravascular thrombosis. This progression is consistent with the report by Rickles and O'Leary of platelet consumption during the initial crisis, leading to the output of more sensitive platelets, etc. Possibly, some patients, such as those studied by Jones, Binder, and Donahoe, might benefit from the early administration of salicylates.

Vascular occlusion is more than ordinarily hazardous to those with sickle trait. Occlusions, which might be survived by persons without the trait, could be complicated by the local sickling of erythrocytes. Thus, individuals with sickle trait might be especially susceptible to thrombotic episodes such as those described by Pirkle and Carstens, who reported six individuals (one male, five females) who died suddenly. Three were known to have experienced some respiratory distress before death and, at necropsy, platelet aggregates were found in the pulmonary arteries and arterioles of all six. These findings are similar to those of Vincent, who described respiratory distress and coma in rabbits after injection of a fat-mobilizing factor isolated from the pituitary. These results strongly suggest that mobilization of fatty acids, which can occur during periods of acute stress, may result in platelet aggregation. Arachidonic acid, a precursor of PGE₂, has been demonstrated to produce this effect. It would be interesting to examine the free fatty acid (FFA) patterns in the blood of individuals who died suddenly. Spector and Hoak claim that arachidonate comprises only a small portion of the plasma FFA content, but this may not be the case in individuals who die of pulmonary embolism during times of acute severe stress.

Thrombosis and sickle crisis are often clinically associated, especially in pregnancy, and contraceptive drugs have also been incriminated in these two abnormalities. Hypercoagulability in pregnancy may result from the release into the circulation of thromboplastic substances from the placenta, but this release is usually associated with abnormalities (e.g., abruptio placenta) that occur in the third trimester. More likely, thrombotic conditions may be caused by an increase in the activation of Fletcher factor (prekallikrein), because the inactivator of complement C₁ esterase, also an inhibitor of Fletcher factor, is decreased during pregnancy and in women taking the Pill. Ratnoff speculates that this failure to inactivate Fletcher factor may contribute to the increased coagulability seen in some pregnant women, and it is tempting to speculate further that thrombosis, leading to microcirculatory occlusion, is partly responsible for the increased tendency (in SCD) toward sickle crises of pregnant women and women taking contraceptive drugs.

Red cells containing HbS, when exposed to hypoxic conditions, may confer an increased viscosity to the blood even before changes in red cell morphology become apparent. Increases in certain plasma globulins may potentiate or exaggerate this effect by augmenting plasma viscosity. For example, Anderson and coworkers recently reported a case of multiple myeloma in a patient who also had SCD. Whole blood from this patient exhibited an abnormally high viscosity when fully oxygenated, and the viscosity was increased further when the blood was treated with dithionite. Washed erythrocytes, placed in plasma not containing the myeloma globulin, conferred no increased viscosity until the oxygen was removed with dithionite. From these and other findings the authors conclude that an interaction between red cells and myeloma globulins, resulting in dramatic increases in blood viscosity in vivo, was responsible for the greater-than-normal severity of SCD in this patient.

Other proteins may affect the blood viscosity. Fibrinogen, a long-chain protein, may exert considerable influence; this protein may increase as much as 500 percent in a variety of chronic diseases or other types of stress. Hyperfibrinogenemia is thought to be a nonspecific reaction to stress; in the rat, at least, the increase can be produced by catecholamine injection: 1 mg/kg epinephrine in oil injected subcutaneously produced, within 24 h, fibrinogen levels about 1.7 times higher than control levels. The response is not affected by simultaneous injections of glucocorticoid and is not produced by corticoid alone, but the adrenal cortex plays a permissive role. Elevations in the fibrinogen level, leading to an increased blood viscosity, may well be an important final common pathway by which chronic stressors influence the severity of SCD or, possibly, evoke crises in persons with sickle trait.

Figure 4 contains a partial summary of those mechanisms known and postulated to influence
the sickle crisis. Though speculative, this diagram seems a reasonable synthesis of the reports cited in this review.

Clinical Tests for HbS. Qualitative tests for HbS are available. Most of these tests depend on reduction of O₂ in blood samples or solutions of hemoglobin; this reduction can be accomplished by incubating blood samples under anaerobic conditions (e.g., sealed cover slip method) or by adding reducing agents, such as dithionite salts. The end point indicating the presence of HbS may be, for example, erythrocyte deformation or a reduction of light transmission. These methods have their applications in mass screening, but they are not adequate for complete qualitative and quantitative evaluation of Hb composition in candidates for aeromedical certification. In terms of complexity and expense, the next most available techniques are electrophoretic; but even these may not be definitive and, as Nalbandian et al. have pointed out (JAMA 234:832–835, 1975), may give misleading results. Therefore, it is probably most economical and prudent to place the responsibility for Hb analysis on hematologists who have the special laboratory facilities and necessary expertise. The Aviation Medical Examiner is, of course, most capable of interpreting lab-
oratory results as they apply to aviation medicine.

Tests of Altitude Tolerance. More useful than Hb analysis would be tests to evaluate the altitude susceptibility of individuals whose red cells contain HbS. As the precautions regarding exposure to high altitude would apply to such clinical tests as well as to physiological training, such tests must be restricted to in vitro and other measurements that can be made on blood samples. A variety of in vitro tests, including measurements of oxygen dissociation properties, viscosity changes, and ionic balance under varying oxygen concentrations, could be appropriate.

Finch and various coworkers have developed an animal model that may be applicable. Ordinarily, human red cells are rapidly destroyed after injection into the circulation of rats, the half-life (T½) for survival being about 15 min; but these authors have reported increases up to 30 h in the T½ of normal human cells in rats pretreated with ethyl palmitate, which blocks the reticuloendothelial system, and a fraction of cobra venom, which destroys complement C3, a component necessary for hemolysis of the foreign cells. Thus, it is possible to study human red cells in vivo without risk to the subject. When rats treated as above are injected with 51Cr-labeled red cells from patients with SCD and subjected to intermittent hypoxia, some of the injected cells disappear from the rat’s blood during periods of hypoxia, only to reappear when normal oxygenation is resumed. Even at normal O₂ concentrations, the survival times of SCD erythrocytes are significantly shorter than those of normal red cells. Cyanate treatment improves survival of the SCD cells when the rats are kept in room air, but no advantage is conferred under hypoxic conditions. Orlin, Castro, and Finch, using a similar model, studied the survival of cells from five donors with sickle trait; the survival times of these cells were unaffected by hypoxia (10 percent O₂) but were shorter under ordinary conditions than those of cells containing no HbS (T½: 19.6 h vs. 34.8 h). The lack of effect on type AS cells by this degree of hypoxia (equivalent to about 18,000 ft) supports our conclusion that moderate altitudes do not produce sickling in persons with sickle trait.

Repetitions of this experiment using a large number of donors of the AS genotype might reveal those very few individuals susceptible to hypoxia. The shorter-than-normal T½ of type AS cells (not attributed by the authors to hypoxia) is also interesting. Perhaps this finding, and the brief survival of red cells in patients with SCD, are related. The extension, by cyanate, of the survival of type SS cells at normal O₂ levels but not in hypoxic conditions supports the speculation that a factor other than HbS may influence survival. Murayama has proposed a second blood factor, in addition to HbS, that influences the sickling process, but the identity of this second factor is still unknown.

The chief obstacle to reaching any definite conclusions about the hazards of aviation to those with sickle trait is the lack of a clear and distinct difference between the heterozygous and homozygous conditions. Erythrocytes of persons with sickle trait can become sickled in vitro if hypoxia is severe enough, and sickled cells have been found in the bodies of persons with sickle trait after sudden collapse and death. In many other inherited diseases, however, the heterozygous condition is free of any signs of disease; for example, heterozygous carriers of Christmas disease (hemophilia B) do not suffer from even mild hemorrhagic disorders, nor have any instances of SUD associated with bleeding been reported in such individuals.

In addition to a slight tendency toward sickling, persons with sickle trait usually have hypostenuria, which also occurs in persons with SCD. Thus, while many abnormal genes do not express themselves at all in the heterozygous condition, sickle trait seems to reveal some of the effects of the Hbβ* gene, possibly reflecting an incomplete, or mixed, dominance in the AS genotype. In addition to the possibility of incomplete dominance, there may be other factors affecting susceptibility to sickling. These are best demonstrated by cases of extraordinarily mild SCD.

No instances of air crashes attributable to sickle crisis have been found in the literature, although with a future increase in the number of pilots of the AS genotype, such reports may appear. If they do, the warnings of Rosenheim and of McCormick should be recalled. There is little oxygen in the blood of a cadaver. With-
out the presence of other significant findings indicating ante mortem sickling—and a history of mild hypoxia is not one of them—a finding of post mortem sickling is of no more diagnostic value than is hemoglobin electrophoresis, and the examiner must content himself with the Scot's verdict: *not proved.* Thus, in the absence of positive history of altitude intolerance or of known contributory factors to such intolerance, persons with sickle trait are probably no more susceptible to hypoxia than are other individuals.

Keeping in mind the possibility that heterozygous individuals may exhibit varying degrees of HbS* gene expression, how may we interpret the conflicting reports reviewed so far? One difference in these reports is apparent: Reports that moderate hypoxia is no threat to persons with sickle trait are based on surveys of large numbers of these individuals who fly, on repeated *in vitro* tests of their erythrocytes, or on complete resistance of test subjects to moderate altitudes. Reports of altitude intolerance, on the other hand, are based on isolated instances of collapse during or shortly after flight. Readers of these anecdotal reports may be provoked to inquire: How many others with sickle trait who were not affected were aboard the same aircraft; what types and amounts of hemoglobin were present in the victim's red cells; and, most important, how many other individuals of this genotype have flown under similar conditions without incident? Persons of the AA genotype may survive hypoxic conditions that lead to unconsciousness; once normal oxygen levels are restored, consciousness is regained and in most cases there is no apparent lasting effect of the hypoxic experience; but in the individual whose cells contain sufficient HbS, an otherwise survivable exposure to hypoxia, if it also leads to sickling, could induce a "vicious cycle" (see Figure 4) of sickling that could lead to death. It must be remembered, however, that, in the SA genotype, initial sickling does not occur (Figure 3) until the oxygen tension approaches 10 mm Hg.

V. Summary and Conclusions.

1. Sickle trait is not a causal factor with respect to altitude intolerance. On the other hand, it may be an important factor in the reversibility of hypoxic collapse.

2. The presence of sickle trait is not, of itself, a basis for aeromedical disqualification, nor is sickling in the cadaver conclusive evidence that a sickle crisis was the primary cause of death or of a fatal accident.

3. It is likely that other factors (e.g., pregnancy, chronic disease, trauma, drug and alcohol abuse) might potentiate the effects of hypoxia in people with sickle trait, and the aviation medical examiner should consider these factors in evaluating individuals with sickle trait for aeromedical qualification.

4. Pilots of the SA genotype should be informed of the probable combined effects of hypoxia and potentiating factors and should be advised that a good oxygen system and its proper use is important to them, not only in preventing sickle crises, but possibly in reversing crises, should they occur.

5. Physiological training, especially in the use of oxygen equipment, is of great potential usefulness to the airman with sickle trait. A hypoxic experience is not necessary for this training and should be avoided. Trainees of the SA genotype should, however, be encouraged to observe hypoxic signs in other trainees who do participate in this phase, as these signs are valuable demonstrations of the insidious nature of hypoxia.

APPENDIX

The author is grateful to Dr. Russell S. Fisher, Chief Medical Examiner for the State of Maryland, for providing five examples of unexpected collapse and death. The cases have not previously appeared in the literature. These individuals were Black, and electrophoretic examination indicated that each was of the AS genotype:

1. A 25-year-old, somewhat obese female, en route to the maternity clinic, collapsed while walking up the hill near a hospital. She was pronounced dead on arrival at the accident room. At necropsy, she exhibited a twin pregnancy and her erythrocytes were markedly sickled. Dr. Fisher writes, "...her Köpfer cells are the most striking example of ingestion of sickle cells that I have ever encountered."

2. A 43-year-old female was "slapped around" in an altercation between 2400 and 0300. She was put to bed and her breathing seemed normal
at this time, but she did not respond to attempts to awaken her. She was pronounced dead at the scene. This individual had a history of bronchial asthma and headaches but no record of heart disease or other illnesses. At necropsy, 80 cc of subdural hemorrhage were found over the left cerebral hemisphere and 20 cc under the left temporal lobe. Although the left hemisphere was shifted somewhat to the right, there were no secondary lesions in the cerebral hemispheres and no lesions of this kind or edema in the pons. Dr. Fisher concluded that these brain changes were not sufficient to explain death. A marked sickling of red cells was seen in all histological sections and there was intense hemorrhage into the splenic follicles. Dr. Fisher concluded that hypoxia or hypotension associated with the original trauma-induced subdural hemorrhage was sufficient to induce a sickle crisis, “which was the true cause of her death.”

3. A 25-year-old male was found dead. He was a known narcotics addict and possessed recent needle marks with subcutaneous hemorrhage. A search of the body for narcotics was without result; blood alcohol was 0.06 gm/dl. “He had all the signs of acute sickle crisis.”

4. A 22-year-old female, a known narcotics addict, was found dead. Analysis of the blood revealed a level of 0.85 mg/dl of a “short acting barbiturate.” There was histologic evidence of sickle crisis.

5. A 26-year-old female exhibited signs of having died in sickle crisis. Blood alcohol was 0.14 gm/dl. A trace of quinine (a common heroin adulterant) was found in the urine, but the results of tests for narcotics were negative.

Dr. Fisher, on the basis of these and other cases, concludes: “I am certainly sufficiently convinced that I would advise any AS hemoglobin individual to avoid hypoxic anesthesia as well as alcohol or drug abuse, or any other situation in which there might be an episode of hypotension or hypoxia, lest an otherwise survivable lesion be suddenly converted to a fatal process.”

References


