Review Article

Sickle cell states and the anaesthetist

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Contents

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Sickle cell disease is still a rare entity for the Canadian anaesthetist to encounter. However, in Montreal and Toronto the black population, which is at high risk for this disease, is estimated by the Canadian Sickle Cell Society to be 120,000 and 300,000 respectively. Given the prevalence of sickle cell disease (SS) at 0.16–1.3 per cent[1] and of sickle cell trait (AS) at eight per cent there are many people at risk in those cities. In many communities outside the metropolitan areas the numbers affected could be so small that some physicians may never see a patient from the population at risk, let alone a patient with proven disease or trait.

Therefore, the purpose of this review is to provide the information for preoperative identification of patients with sickle cell disease or trait. Clinical indicators and tests to confirm the diagnosis will be detailed. Preoperative treatment of these patients, with current views on transfusion therapy, will be discussed. Finally, management of anaesthesia will be suggested.

Characteristics of the disease

History

Sickle cell disease was first described by a Chicago physician, Herrick, in 1910.[2] He observed the characteristic sickle-shaped cells in the peripheral blood film of a black student with recurrent bronchitis, cervical lymphadenopathy, skin ulceration and anaemia. Scriver and Waugh, in 1930, reported the first case of sickle cell anaemia in Canada and made important observations on *in vivo* sickling and its reversibility with oxygenation of blood.[3] The disease is now known to be hereditary and the result of a genetically determined haemoglobinopathy.

Epidemiology and genetics

The sickle cell gene (S gene) has a worldwide distribution but is most concentrated in West Central Africa, the northeast corner of Saudi Arabia and East Central India. The sickle cell gene in North America was probably introduced as a result of West African immigration.[4] The most widely accepted theory of the sickle gene origin is that it originated from identical mutations in different areas of the world.[5] Geographical studies of deoxyribo-

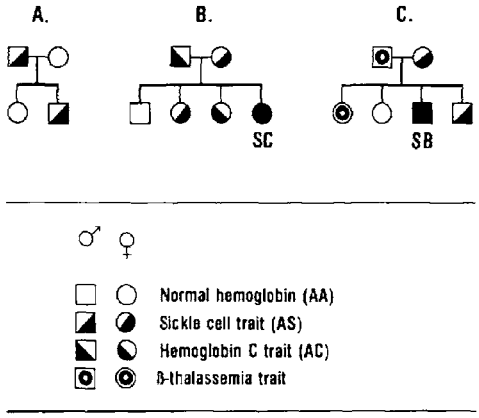

FIGURE 1 Inheritance of sickle cell disease: various possible matings – SC, SBeta refer to the sickle cell diseases SC and SBeta thalassemia. Mating A is not at risk to produce a child with sickle cell disease whereas B and C are.

nucleic acid (DNA) polymorphisms of the S gene show that it arose separately in Ghana (formerly Nigeria) and from Central and East Africa. The S gene in Arabia and India probably spread from East Africa. However, it should be emphasized that the gene is not confined by race or skin colour. It is found in southern Italy, northern Greece, southern Turkey and has been reported in Caucasians and other populations.[6]

Association with malaria

The distribution of sickle cell disease parallels that of falciparum malaria in the old world. Infants with sickle cell trait are partially protected against plasmodium falciparum, so they become infected less frequently and have milder infections.[7] Parasitized sickle trait cells are more rapidly cleared by the reticuloendothelial system than nonparasitized cells but the exact cellular mechanisms require further clarification.

Inheritance

The inheritance of sickle haemoglobin follows Mendelian laws to an extent. Homozygotes (sickle cell disease or SS patients) will show clinical symptoms of the disease whereas heterozygotes (sickle cell trait or AS patients) are asymptomatic. Haemoglobin A and S genes have codominant expression resulting in the production of normal and abnormal haemoglobin in the same patient. Matings where there is a risk of producing a child with sickle cell disease include AS with AS, AS with AC (haemoglobin C trait), and AS with AB (beta-thalassemia trait). If both parents' genotypes are known, then genetic counselling is possible. The results of some common matings are illustrated in Figure 1. It is important to screen both parents as one partner may have no haemoglobin S but possess haemoglobin C trait or beta-thalassemia trait. The offspring would then be at risk for sickle cell disease if the other partner is heterozygous for haemoglobin S (AS, SS, or SC).

Clinical features

Sickle cell disease produces clinical symptoms of variable severity. However, sickle cell trait, the carrier state, is almost completely benign, requiring extremely hypoxic or acidotic conditions before sickling occurs.[8,9] Homozygous sickle cell disease (SS) or the doubly heterozygous states (SC, S beta-thal) produce chronic incurable illness with unpredictable life-threatening events. Children less than six months old show fewer clinical manifestations because of the protective effects of fetal haemoglobin, but under adverse conditions sickling can occur even in the newborn.[10]

The shortened red cell survival time, with a lifespan of approximately 12 days, causes anaemia and hyperbilirubinaemia due to haemolysis. As in other chronic haemolytic anaemias the patient is at risk for early development of gallstones and aplastic crises. Compensatory erythropoeisis in the bone marrow produces a reticulocytosis and bony enlargement of the skull and long bones, with a characteristic prominence of the forehead. Hepatosplenomegaly and a painful dactylitis, the hand-foot syndrome, are manifest in young patients along with the sequestration syndrome and an inordinately high risk of overwhelming sepsis.[11,12] Morbidity and mortality result from cerebrovascular accidents,[13,14] vitreous haemorrhages, retinal detachments,[15] cholelithiasis,[16] priapism,[17] leg ulcers,[18] avascular necrosis of bone[19] and the acute chest syndrome caused by pulmonary infarcts.[20] The risk of these complications varies somewhat with age but most tend to occur in an unpredictable fashion.

A distinctive feature of the clinical course is the ischaemic pain crisis. These crises are vaso-occlusive events which result from the occlusion of blood vessels by clumps of sickled cells, causing tissue anoxia. They are exacerbated by exertion, infection, dehydration, cold, hypoxaemia or vascular stasis. The pain is often sufficiently severe to warrant hospitalization and the administration of narcotic drugs. In a similar manner autosplenectomy can occur, although compromised splenic function is apparent as early as four months.[21] Aplastic crises with severe anaemia can be fatal, and are often due to marrow suppression secondary to parvoviral infections.[22] Haemolytic crises, with further exacerbation of the anaemia and reticulocytosis are seen after acute infections, possibly more often in patients with glucose-6-phosphate dehydro-

genase deficiency (G6PD).[23] The sequestration crises, with sudden pooling of the blood volume in the spleen, occur more frequently in infants and children[12] and is an important cause of death in that age group. Immediate transfusion is the treatment of choice and elective splenectomy should be considered for recurrent episodes.[12,24]

Modifiers of clinical severity

Although the disease is generally severe, there is important variability in severity that as yet eludes complete explanation or accurate assessment. Long survival without serious complications is possible. Relevant genetic factors include the presence of alpha-thalassemia and the level of haemoglobin F.

Alpha-thalassemia interacts with sickle cell disease to reduce the rate of haemolysis, increase the haemoglobin and increase the deformability of red cells.[25] Splenomegaly is more common in patients with alpha-thalassemia while the acute chest syndrome and leg ulcerations are less. There are conflicting reports of the effect of alpha-thalassemia on prolonging survival.[25]

Persisting high levels of haemoglobin F ameliorate the clinical and haematological features of sickle cell disease.[26] In the Eastern Province of Saudi Arabia sickle cell disease is clinically benign because of high HbF levels and the prevalence of alpha-thalassemia in that population.[27]

Pathophysiology of the disease

Haemoglobinogathy

Pauling *et al.* in 1949[28] recognized that sickling is a reversible change in shape of the red blood cells, from the normal biconcave discs to irregular forms, in response to lowering of the partial pressure of oxygen. They showed that this only occurred in erythrocytes containing an abnormal haemoglobin (HbS), which could be distinguished from normal haemoglobin (HbA) by its electrophoretic mobility. The abnormality in sickle cell disease was identified by Ingram in 1957[29] as a substitution of glutamic acid by valine in the sixth N-terminal amino acid position on the beta chain of the globin molecule. The haem group is the same in both proteins, as are the two alpha chains. The major hallmarks of this disease, the haemolytic anaemia, the red cell shape change and the vasoocclusive events can be explained on the basis of the abnormal haemoglobin.

The anaemia in sickle cell disease is caused by accelerated red cell destruction with measured red cell survival times as low as 15 per cent of normal.[30] Factors which are relevant to this extravascular haemolysis include decreased cell deformability that allows plugging of small vessels,[31] the increased adherence of sickle cells to vascular endothelium,[32] spontaneous autooxidation[33] and the formation of dense cells with very short lifespans.[34] There is also evidence that erythropoietin response to the anaemia is blunted, preventing adequate bone marrow compensation.[35]

The single nucleotide change that gives the amino acid substitution in the globin chain of haemoglobin is responsible for the important fundamental event of polymerization (the alignment of haemoglobin into fibres along the long axis of the red cell) with deoxygenation.[36] Before the erythrocyte changes shape this phenomenon is the start of gel formation, which is the loss of haemoglobin solubility. Both ultrastructure and solubility data favour the model of a 14-strand fibre as the predominant formation in both red cells and free solution. The fibre is aligned in a way similar to a right-handed helix of high pitch with a periodicity of 3000 A.[37]

The formation of deoxyhaemoglobin S is a prerequisite for polymerization. Polymerization is influenced by pH, temperature, the concentration of haemoglobin and oxygen saturation.[38] The presence of haemoglobin F increases the minimum concentration of total haemoglobin required for gelation although it does not participate in sickling.[39] Mixtures of haemoglobins S and A have lower solubilities than comparable mixtures of haemoglobin S and F. It can be shown that haemoglobin C participates in the same way as haemoglobin A in the gelling process.[40]

After the induction of polymerization there is a delay time which is followed by the sudden formation of polymer. The delay time is markedly influenced by small changes in haemoglobin concentration. An increase in haemoglobin concentration can shorten the delay time considerably, leading to a faster rate of polymerization.[41] The most dense cells with the highest mean corpuscular haemoglobin concentration (MCHC) are most susceptible to fibre formation and decreased deformability. Whether the cells cause vascular occlusion depends on the rate of polymerization. Factors which affect the delay time include the concentration of haemoglobin F, the capillary blood flow rate and the rate of oxygenation.[42]

Oxygen affinity

Sickle cell blood has a lowered oxygen affinity because of this intracellular polymerization of haemoglobin S,[43] but the high level of 2–3 diphosphoglycerate (2.3-DPG), resulting in a higher $P_{50}O_2$, also plays a role. Thus, the oxygen dissociation curve is shifted to the right. This facilitates oxygen delivery to the tissues to compensate for the anaemia but it also promotes the formation of deoxyhaemoglobin and further polymerization. When pH is lowered, such as physiologically in the capillary bed, this is further enhanced. As fetal haemoglobin (HbF) increases oxygen affinity, the oxygen dissociation curve

shifts to the left, lowering the $P_{50}O_2$. The presence of HbF in the first few months of life appears to compensate partially for the effects of HbS.

In vitro studies show that intracellular polymerization starts when the oxygen saturation is below 85 per cent (corresponding to an arterial PO_2 of 40–50 mmHg) and is complete at 38 per cent saturation in sickle cells (SS).[44] Therefore, patients with sickle cell anaemia are constantly sickling because the PO_2 of mixed venous blood is 40 mmHg, which is their critical level, while individuals with sickle cell trait are normally not at risk. Sickle trait cells (AS) do not sickle until oxygen saturation is below 40 per cent (arterial PO_2 of 25–30 mmHg).[44] Cells are heterogeneous with respect to the amount of polymer formed at varying levels of oxygen saturation.[45] Nuclear magnetic resonance (NMR) studies showed that the most dense cells, with the highest MCHC, form significant polymer even at high oxygen saturations.[45]

Blood viscosity

The change of haemoglobin solution to a gel increases viscosity which, in this non-Newtonian fluid, is very dependent on shear rate. The latter also can lower the delay time to aggravate vasoocclusion and have the opposite effect of increasing viscosity. This decreases the risk of vessel obstruction.[47]

Cell membrane damage

There is a role for membrane damage in the pathogenesis of the disease.[47] The irreversibly sickled cells which are very rigid with short lifespans contribute to vasoocclusion and high viscosity. Their numbers are not constant over time and do not consistently relate to clinical severity.[48] They contribute to vasoocclusion more by their high haemoglobin concentration than their damaged membrane. Abnormalities in the membrane which probably are relevant include the membrane loss that occurs with deoxygenation,[49] the change in phospholipid orientation (or "flip flop"),[50] and oxidant damage to the membrane.[50]

Sickle cells have an increased adherence to the endothelial surface of blood vessels,[32] which may be related to the fact that phosphotidyl serine and phosphotidyl ethanolamine are found on the outer surface of the lipid bilayer in deoxygenation.[50] This "phospholipid flip flop" can enhance procoagulant activity.[52]

Microcirculatory changes

Events in the microcirculation are likewise important[53] but less is known about normal regulation of blood flow, oxygen transport and oxygen unloading.

Molecular biology of sickle cell anaemia

Technology permitting deoxyribonucleic acid (DNA) analysis has changed our concept of genetic diseases, including sickle cell disease, and has led to important practical applications in anthropological studies and prenatal diagnosis. Haemoglobin S differs from haemoglobin A by the substitution of the amino acid valine for glutamic acid at the sixth N-terminal amino acid of the beta globin chain of the haemoglobin molecule. Haemoglobin S is an abnormal product of a synthetic process that originates in the DNA of the globin gene which is located on chromosome eleven. The information in the genetic DNA is relayed by messenger ribonucleic acid (mRNA) to the cytoplasm where protein synthesis (the synthesis of the globin chain) occurs. The information for the structure of haemoglobin S is contained in the nucleotide sequence of the DNA. For haemoglobin, three nucleotide bases or a triplet codon[54] specifies the amino acid assembled at position six of the beta globin chain. The single base mutation (A to T or adenine to thymine) in this triplet codon accounts for the synthesis of haemoglobin S instead of haemoglobin A.[55]

Enzymes derived from certain bacteria can be used to split DNA into various fragment lengths. Using the Hpa I enzyme, nearly all Caucasians and 97 per cent of blacks with only haemoglobin A (AA) have 7.6 kb fragments that encompass the beta-globin gene. American blacks with haemoglobin S have the 13.0 kb fragment.[56] Geographical studies of linkage patterns show the S gene

TABLE I Diagnosis of sickle cell states

Method	*Comments*
Clinical examination	Often unrewarding and lacks precision.
Complete blood count with reticulocyte percentage	Values may be normal in some sickle cell diseases.
Peripheral blood film examination	Needs an experienced observer.
Haemoglobin electrophoresis	Cellulose acetate electrophoresis initially. If an abnormal band is found, its identity is confirmed by electrophoresis using citrate agar. These are the only tests useful in newborns and infants. They are standardized, simple and cheap.
Sickle cell preparation (metabisulphite) or sickledex	Do not use for screening. These do not distinguish sickle cell trait from the sickle cell disease. Nonspecific tests and inaccurate in the first two years of life.
Other methods	These are less commonly used but show promise. Include thin layer isoelectric focusing, high performance liquid chromatography and the use of monoclonal antibodies.

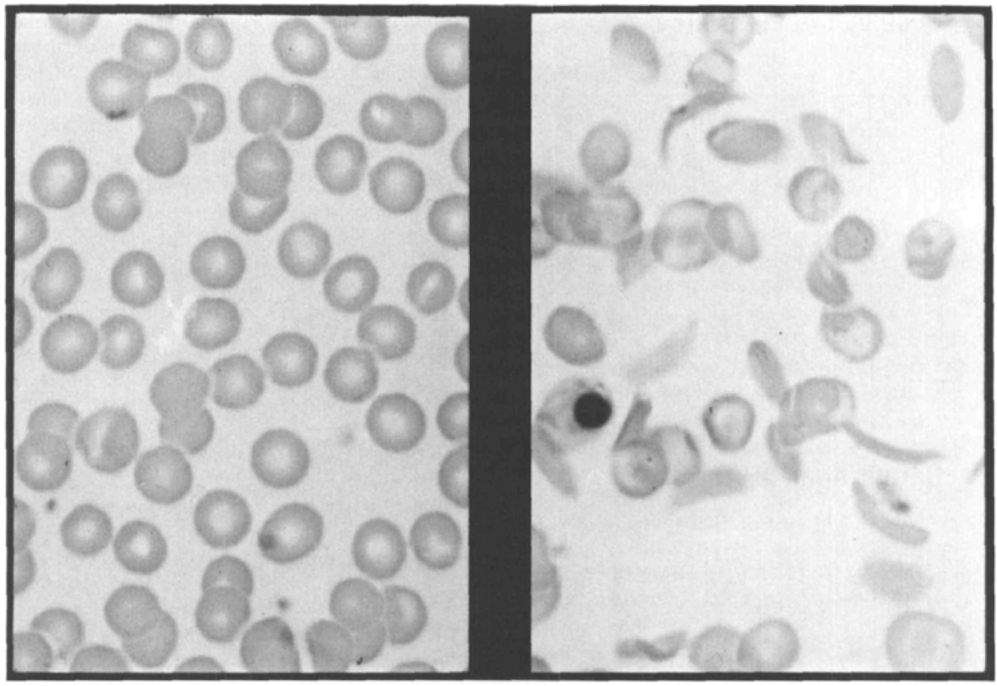

FIGURE 2 Sickle cell trait and sickle cell anaemia in the peripheral blood film – *Left panel*: sickle cell trait. Essentially normal morphology is illustrated. *Right panel*: sickle cell anaemia (SS). This is the child of the mother in left panel. Note nucleated red blood cells, target cells and sickle forms.

is linked to the 13.0 kb fragment in Americans with origins in West Africa, Algeria and Morocco, Togo and Sicily.[57] The gene is linked with the 7.6 kb fragment in Gabon, the Ivory Coast, Kenya, Saudi Arabia and India.[58] These restriction fragment length polymorphisms (RFLPs) are associated with the S gene and support the theory that there were multiple independent S gene mutations in various populations in Africa.[59]

Diagnosis

Clinical examination

Some children and infants may present with a normal physical examination and a history of previous blood testing for abnormal haemoglobin should be sought. Some clinical signs can give nonspecific clues to the diagnosis. There may be signs of anaemia or scleral icterus. Frontal bossing and growth abnormalities may be present. The liver may be felt but the spleen is seldom palpable over the age of five years. Precise diagnosis depends on laboratory testing (Table I).

Peripheral blood film and CBC

A careful clinical evaluation combined with a review of the complete blood count (CBC and reticulocyte percentage) and peripheral blood film will confirm sickle cell disease if sickle forms are seen on the blood film. Unfortunately, the number of sickle forms varies considerably so they can be hard to find. Sickle cell trait patients do not show sickle forms on the peripheral blood film and may have essentially normal red cell morphology (Figure 2). Patients with sickle cell disease of the SC type may have normal blood counts. Unless the reticulocyte count is elevated for the level of haemoglobin and the abnormal red cell morphology is appreciated, the diagnosis may be missed.

Haemoglobin electroghoresis

Specific diagnosis of the haemoglobin type is made only by haemoglobin electrophoresis (Figure 3) and it should be repeatedly emphasized that this is the only way of diagnosing the haemoglobinopathy accurately. All other methods have shortcomings and do not suffice for a

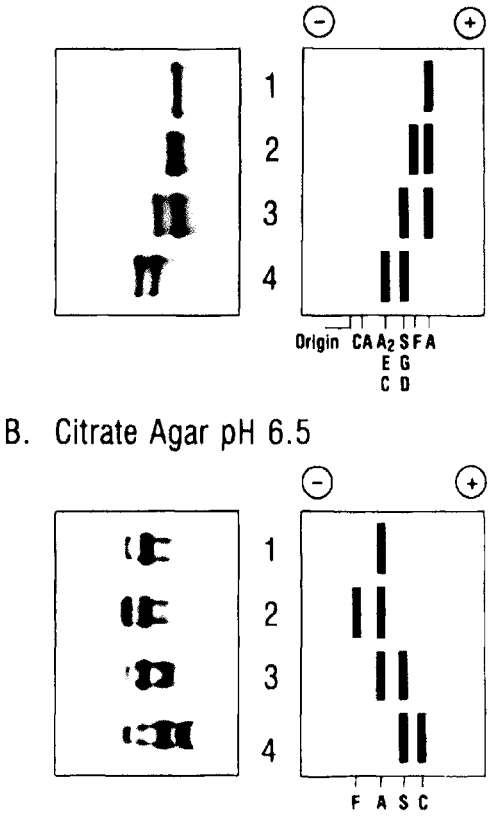

FIGURE 3 Haemoglobin electrophoresis: cellulose acetate and citrate agar – The figures on the left are photographs of the actual laboratory tests whereas those on the right are schematic representations of the photos. The numbers 1–4 refer to different patients, each tested by cellulose acetate and citrate agar. Patient 1 shows a normal adult pattern (AA). Patient 2 is a newborn (FA). Patient 3 has sickle cell trait (AS). Patient 4 has sickle cell disease of the SC type (note that there is no HbA).

screening program, whether for adults, infants or newborns.

Emergency use of sodium metabisulphite preparations

Diagnosis in an emergency situation can be challenging because a consultant haematologist may not be available and haemoglobin electrophoresis results cannot be obtained immediately. Reliance has to be placed on the history and physical examination, complete blood count (CBC), reticulocyte count and examination of the peripheral blood film. In older children and adults, a sodium metabisulphate preparation can also be helpful (Appendix I). If this is negative at one hour, the patient does not have haemoglobin S. If positive, scrutiny of the CBC may show normal blood values suggesting trait, or indicate anaemia and reticulocytosis indicative of sickle cell disease. Sickle forms, even in small numbers, are diagnostic of sickle cell disease and are not seen in sickle cell trait.

Neonatal diagnosis

The diagnosis in newborns can now be made accurately by routine electrophoretic methods.[60,61] There is evidence that early diagnosis may be lifesaving in children with sickle cell disease,[62] so diagnostic testing is important. Earlier methods were confounded by the problem of reliably determining the presence of haemoglobin A when haemoglobin F is present in the large quantities seen at birth. On cellulose acetate electrophoresis (Appendix I) the wide band of haemoglobin F may obscure the A band just anodal to it (Figure 3). In infants with an SF pattern demonstrated on cellulose acetate, citrate agar electrophoresis can confirm the presence of haemoglobin A (Appendix I). Infants with small amounts of haemoglobin S and predominantly haemoglobin F may have haemoglobin SS. Those in whom some haemoglobin A is identified may have sickle cell trait (AS) but need further family studies to rule out sickle thalassemia. It should be noted that other haemoglobins besides S migrate to the "S" position on cellulose acetate electrophoresis and these can be separated by citrate agar electrophoresis. Likewise, haemoglobin C, E and O all run in the A2 band on cellulose acetate and need further elucidation by citrate agar electrophoresis. Cellulose acetate electrophoresis with citrate agar confirmation is now the standard method for sickle haemoglobin identification in the newborn period. It is simple, cheap and standardized with reliability to detect haemoglobins S, C and A even in the presence of large amounts of haemoglobin F.

Prenatal diagnosis

Accurate methods are also available for the prenatal diagnosis of sickle cell anaemia. Now that the restriction enzyme MstII, which is specific for the sickle mutation[63] is available, sampling of fetal blood is unnecessary and amniotic fluid samples can be used. Very early prenatal diagnosis is possible by analysis of DNA from chorionic villi.[64]

Treatment

Supportive

There is as yet no cure for this disease. Patients need close follow-up in centres which can manage the complications

of the disease, provide health maintenance and comprehensive care. Counselling and education are very important in helping families to cope and accept this chronic illness no matter how mild it may be in certain patients. Educational needs change with age and sickle cell centre staff must be aware of these changing needs as children grow and develop. Precautions against infections, prophylactic penicillin and information on splenic sequestration are vital components in teaching mothers of young infants. Pain management and eventually birth control, education and employment opportunities become as relevant as coping with the effects of the end organ damage of this disease.

Since this is a chronic haemolytic anaemia, most patients receive folic acid supplements, although the absolute need for them in every patient is not proven.[65] Pneumococcal and haemophilus influenza vaccines are given at two years[66,67] as well as all other routine immunizations. Influenza vaccine is also recommended and children on transfusion programs receive the hepatitis B vaccine. All children receive prophylactic penicillin from four months and continue until seven years,[62] although the precise age of discontinuation remains unproven and controversial.

Routine monitoring for end organ damage may include regular ophthalmology examinations, dental examinations, periodic urinalyses and renal function tests and ultrasound examination for cholelithiasis. During periods when the disease is stable, clinical and laboratory parameters should be documented for comparison with the results of testing during a crisis or complication of the disease.

Specific therapies

Medical management of this incurable hereditary disease remains primarily supportive and symptomatic, although many specific treatments have been suggested. These have been reviewed in detail recently by Aluoch.[68] Early approaches included the use of splenectomy, liver extracts, drugs and fluids such as iron, sodium bicarbonate and thiocyanate, blood transfusions, oygen, vasodilators, alkalis, anticoagulants, carbonic anhydrase inhibitors, phenothiazines, plasma volume expanders and steroids (mainly testosterone). More modern approaches, with some scientific basis, included gelation inhibitors such as urea, cyanate and the synthetic vasopressin analogue, desamino-D-arginine vasopressin (DDAVP) and cell membrane modifiers such as zinc, procaine hydrochloride, citiedil, and piracetam. Most of these have proved disappointing.[69]

The first of several clinical trials with urea was in 1971: an attempt to disrupt hydrophobic bonds and unsickle red cells. No benefit in treating or preventing crises was found. Cyanate proved to be too toxic for use, and was also ineffective. With the usual oral doses, extracorporeal cyanate is much less toxic and, although it is impracticable for routine practice, has not been completely discarded.

DDAVP and a high fluid intake were used to decrease the concentration of haemoglobin S (or decrease the MCHC) by increasing the red cell volume through hyponatremia. There was a significant effect with a reduction in the number of crises and little associated toxicity but strict adherence to the treatment regimen was essential.[70] The precise role of zinc needs further evaluation.[71] Cetiedil, a membrane modifier originally used as a vasodilator, was found to be beneficial at doses of 0.4 $mg \cdot kg^{-1}$ in reducing the number of painful sites and shortening the total time in crisis.[72] No serious adverse reactions were noted. This drug is not generally available and its benefits in sickle cell crises have not yet been confirmed.

Splenectomy is now part of the management of some children with splenic sequestration crises.[24] Blood transfusions can be specifically indicated but also have a definite role in treatment in this disease. Oxygen therapy is only used when the patient is hypoxaemic and bicarbonate to treat acidosis. Embury *et al.*[73] have carefully evaluated oxygen therapy and recommended that oxygen should be administered intermittently, rather than constantly, to avoid the rebound increase in irreversibly sickled cells seen after discontinuing continuous oxygen inhalation.

Augmentation of haemoglobin F

Recent proposals for management are aimed at being curative and include augmenting haemoglobin F production and bone marrow transplantation.[74–77] It is probably only a matter of time before specific gene therapy is also considered.[78] Haemoglobin F has a role in modifying sickle cell disease. *In vitro*, haemoglobin F inhibits the polymerization of haemoglobin S. Clinically, patients with pancellular hereditary persistence of fetal haemoglobin (PHPFH) are asymptomatic and Saudi Arabians with high haemoglobin F levels have relatively mild disease. Platt[76] has reviewed the chemotherapeutic agents, particularly 5-azacytidine and hydroxyurea, which can increase the haemoglobin F level in patients with sickle cell disease. The precise mechanisms are not known but inhibition of methylation of the gamma globin gene may be involved. In some patients these therapies have been beneficial but the long-term side effects and toxicities are not known.[79,80]

Bone marrow transplantation

A recent report of a bone marrow transplantation in a child with acute myeloblastic leukaemia and sickle cell anae-

mia demonstrated the significant toxicity of the treatment, but also the possibility of cradicating the leukaemia and the sickle cell anaemia.[77,81] Complications included acute graft-versus host disease (GVHD), femoral head necrosis and pneumococcal sepsis. Usually transplantation is restricted to patients who have an identical HLA matched, MLC nonreactive sibling donor. This restricts the number of donors and GVHD also causes significant morbidity. The unpredictable nature of sickle cell disease makes it impossible to determine which infants will eventually have frequent vasoocclusive crises or strokes and thus be classified as severe. By the time the clinical pattern is obvious the child may have received many transfusions making transplantation difficult. The patients with severe disease may be those most likely to benefit but at present there is no role for bone marrow transplantation in sickle cell anaemia alone.

Transfusion therapy

Sickle cell anaemia is a chronic haemolytic anaemia but transfusion should only be used when specifically indicated. Blood is given to improve oxygen carrying capacity and transport and to reduce the proportion of haemoglobin S. Simple transfusion is not indicated in chronic steady state anaemia but may be given to treat severe anaemia even with high output cardiac failure, postural hypotension or cerebral symptoms. These might occur during an aplastic crisis or with splenic sequestration. Hypoxaemia which occurs with pneumonia and pulmonary infarction would also warrant transfusion.[82]

Partial exchange transfusion reduces the percentage of erythrocytes containing haemoglobin S and improves microvascular perfusion. This is indicated in life-threatening infections, cerebrovascular accidents, splenic or hepatic sequestration, intractible priapism, progressive pulmonary disease and fat embolization.[83] Partial exchange transfusion is sometimes recommended before intra-arterial contrast injection,[82] as preparation for general anaesthesia and to aid healing of ulcers.[84] The use of transfusion therapy during pregnancy and to terminate prolonged painful crises is more controversial.[85]

In areas where sickle cell disease is endemic donor blood should be screened routinely for sickle haemoglobin. The crossmatch and screen for alloantibodies is otherwise the same as for normal patients. The use of packed red cells is standard therapy. Most circumstances require a partial exchange transfusion that will rapidly decrease the number of sickle cells to less than 30 per cent (monitored by electrophoresis) without increasing the haematocrit to more than 36 per cent.[86,87]

Simple transfusions may be used in some situations,[88] depending on the baseline haemoglobin. A patient with a steady state haemoglobin of 60 $g \cdot L^{-1}$ may decrease the percentage of S-cells to 45 per cent by simple transfusion to 120 $g \cdot L^{-1}$. A patient with a baseline haemoglobin of 100 $g \cdot L^{-1}$ (as seen in SC type sickle cell disease) will need phlebotomy and a partial exchange transfusion to achieve a lowered percentage of HbS without overtransfusion and the risk of hyperviscosity.

The complications of blood transfusion are the same as for other patients. Alloimmunization is a particular problem[89] and the high frequency may derive in part from the use of racially mismatched blood.[90] Infectious complications such as acquired immune deficiency syndrome (AIDS) and hepatitis or iron overload may occur.[91] Severe delayed tranfusion reactions have occurred,[92] further discouraging the unnecessary use of blood transfusions.

Organization of sickle cell centres in North America

The United States Congress passed the National Sickle Cell Anemia Control Act in May 1972. This helped to establish a sickle cell disease branch in the Heart, Lung and Blood Institute of the National Institutes of Health (NIH). From this beginning ten comprehensive centres to care for patients with sickle cell anaemia were founded. In 1977 The National Heart, Lung and Blood Institute of the NIH sponsored and supported a multi-institutional clinical study, "The Natural History of Sickle Cell Anemia." Most projects were completed in 1987 and resulted in many useful publications on sickle cell anaemia in patients from birth to adulthood.[93,94]

In Canada there is a smaller population at risk, as well as less public awareness of the disease. There is no Canadian legislation comparable to that in the United States. The Federal Government (through the Bureau of Epidemiology of the Laboratory Centre for Disease Control) has a voluntary Congenital Anomalies Surveillance System which could encompass sickle cell anaemia and thalassaemia. Until now, the Canadian Sickle Cell Society has been responsible for most of the community education in this country. This Society was organized in 1976 and has branches in Montreal, Toronto, Halifax and Calgary which provide both community and health professional services. Screening and counselling for the community are available on request as well as information for health professionals.[95]

At present, the major organized paediatric programmes for sickle cell disease are in Montreal (Ste. Justine Hospital and The Montreal Children's Hospital) and Toronto (Hospital for Sick Children). An adult programme has also been started in Montreal (Royal Victoria Hospital). The locations of those centres reflect the larger population at risk in these urban areas.

Management of general anaesthesia

Anaesthesia for patients with sickle cell states has been the subject of a number of excellent reviews over the last

twenty years.[96–102] Recommendations for management have been according to the changing knowledge of the pathophysiology of the condition and prevailing economic conditions. Increasing surgical demands for early correction of cardiac anomalies and major surgery throughout life should now be met confidently in these patients. However, it is difficult to separate the complications of the disease process from those resulting from anaesthesia and surgery, while the potential benefits of transfusion techniques need to be assessed against the growing awareness of their risks. In the context of North American practice, sickle cell disease is essentially a haematological problem, so that anaesthesia is incidental to the long-term management of the patient.

Preoperative considerations

At the time of preoperative examination all patients at risk should be referred to a haematologist for diagnosis and assessment of their current status (Appendix II). The decision for blood transfusion needs to be made and the technique determined. Particular attention should also be given to the presence of pre-existing pulmonary hypertension or cardiomegaly, which are complications of sickle cell disease. Chest *x*-ray is indicated in adults as cardiomegaly is often present. In older adults pulmonary hypertension and cor pulmonale develop as a result of repeated pulmonary infarctions.

The preparation of the patient with sickle cell disease includes the administration of folic acid and consideration of prophylactic antibiotics, depending on the procedure. Hypovolaemia must be avoided to prevent dehydration, hyperosmolality and increased viscosity, by giving intravenous fluid therapy during the period of preoperation starvation. Premedication can be avoided to prevent cardiorespiratory depression, but promethazine (1 $mg \cdot kg^{-1}$ orally two hours before surgery) may be a useful drug because of its possible inhibition of sickling of deoxygenated HbS.[103]

Technique of anaesthesia

The same considerations apply to all sickle cell states when anaesthesia is contemplated. Although sickle cell trait patients can usually be treated as healthy with regard to anaesthesia and surgery, there are exceptional circumstances where they are also at risk of sickling.[8,9,104] Severe physiological stress due to extremes of hypoxia, hypothermia or acidosis is needed before they are in jeopardy. Under such conditions, normal tissue might also be expected to be vulnerable. Sickle cell trait individuals have developed sickle cell crises during endurance training.[8] During anaesthesia involving a difficult intubation, delivery by Caesarean section,[104] massive blood transfusion or cardiopulmonary bypass similar circumstances can be foreseen.

The choice of anaesthetic technique is less important than the care with which it is administered. Either regional or general anaesthesia may be used, as long as there is meticulous attention to details of patient care. Local infiltration and regional methods are often used and spinal, epidural, and caudal anaesthesia have been used successfully for surgery, postoperative analgesia and to eliminate the pain during a crisis. However, hypotension can accompany these techniques so adequate volume replacement is essential. Restoration of blood pressure with fluid loading is preferable to having to resort to pressor agents which can produce vasoconstriction and stasis. The use of tourniquets may produce local stasis and acidosis on cuff deflation. An increase in serum lactate and venous carbon dioxide tension has been demonstrated[105] and hyperventilation may be beneficial in counteracting these effects. Careful exsanguination of the limb and the shortest possible time for cuff inflation is also suggested.

Important considerations include the maintenance of oxygenation with monitoring of oxygen delivery and oxygen saturation of the patient throughout the procedure. The use of preoxygenation before induction may minimize the dangers from airway obstruction or an unexpectedly difficult intubation, and inhalation of 30–50 per cent oxygen for 24–48 hrs postoperatively[98] should guard against hypoxaemia in the recovery period. Complications are more common following surgery than intraoperatively so observation into the recovery period is essential, precluding discharge on the same day as surgery. Chest physiotherapy should be instituted as early as possible with early ambulation. Hydration must be maintained and further transfusion may be required if the HbS concentration begins to rise.

Temperature homeostasis should be maintained agressively using heating blankets, warmed intravenous fluids, humidification of inspired gases and a warm ambient temperature. Acidosis due to hypoventilation, massive blood transfusion or pooling of blood in dependent parts of the body may compromise these patients. Therefore, monitoring of end–tidal carbon dioxide levels and arterial and venous blood gas and acid–base status intermittently is important. Appropriate early administration of intravenous sodium bicarbonate, or adjustment of ventilation can then be undertaken.

Sickle cell crisis intraoperatively

The diagnosis of a sickle cell crisis intraoperatively is the diagnosis of organ dysfunction due to vaso-occlusion by sickled cells. This is difficult as the clinical signs are masked by general anaesthesia. In a patient who has been transfused preoperatively, it is assumed that vaso-occlusion is less likely to occur and infective complications are more likely to be the presenting signs.

Convulsions or a change in the breathing pattern may be noticed in a patient during spontaneous respiration, a change in blood pressure or haematuria may also accompany a crisis. If a muscle relaxant has been administered and intermittent positive pressure ventilation instituted then even these signs may not be evident. As infarctive crises are the most common,[98] pulmonary infarction is one of the few clinical sequelae which may be identified easily. Postoperative analgesia may diminish pain due to headache or abdominal crises, priapism, hand-foot syndrome or musculo-skeletal pains, or these may be confused with the effects of anaesthetic agents. Signs relating to the central nervous or respiratory systems may be more likely to be detected. Treatment of a crisis during and after surgery involves the transfusion of fresh packed red cells to reduce the percentage of HbS, or the use of antibiotics to treat infections aggressively. A peripheral blood film and reticulocyte count is not helpful in sickle cell disease patients in whom some sickled erythrocytes are always present. Essentially, the diagnosis is made by alertness to symptoms of organ dysfunction due to vaso-occlusion and infection.

Controversies in anaesthetic management

Preoperative use of blood transfusions

There has been no prospective analysis of the effect of preoperative blood transfusion on the outcome of general anaesthesia and surgery in patients with sickle cell anaemia. Correction of anaemia and a reduction in the percentage of circulating HbS restores the patient's homeostasis and aims to reduce the operative risk to normal. It is not clear whether sickle cell disease is associated with an increase in postoperative complications or whether some procedures predispose more to problems than others. Indications for preoperative transfusion may be limited to certain types of surgery. Patients with sickle cell trait do not appear to need transfusions but patients with sickle cell disease cause more concern, so reports of deaths of untransfused patients after minor surgery are difficult to ignore.[106,107] Some relevant studies which are frequently quoted provide the framework for considering the risks and benefits involved in transfusing these patients for general surgery. These studies will now be reviewed.

In 1969, Holzmann *et al.*[108] reported the Brooklyn Hospital experience (1956–1967) which covered 112 operations in 93 patients aged 13 months to 52 years. Of these, 47 patients with sickle cell trait demonstrated no intra- or postoperative complications. The 46 patients with sickle cell anaemia (SS in 43, SC in two, S-Thal in 1) were undergoing cholecystectomies or minor gynaecological surgery under regional or general anaesthesia. Of these, five had severe postoperative complications with one pulmonary infarction, three cases of pneumonia and a death from septic endometritis. The use of blood was conservative and the adult patients were transfused only if the preoperative haemoglobin was below 70 $g \cdot L^{-1}$ but the children were transfused liberally, although no preoperative exchange transfusions were given.

Searle[99] in 1973, reviewed the reports of anaesthesia in sickle cell states and concluded that, at that time, patients with sickle cell trait showed no increase in complications. In the sickle cell anaemia patients reported from 1955–1972, there were 11 deaths in 144 operations, of which five might have been unavoidable. He advocated conservative use of blood, transfusing only when the haemoglobin was below 50 $g \cdot L^{-1}$

The Jamaican experience is often cited for its notable lack of the use of transfusions to prepare for surgery. Homi *et al.*[109] reported their experiences (1958–1978) with 200 patients undergoing 284 operations. The patients' ages ranged from two months to 69 years, with a mean of 19 years in the patients with sickle cell anaemia. The mean haemoglobin of the 159 patients who did not receive blood preoperatively was 82 $g \cdot L^{-1}$. Postoperatively, 137 of the patients were managed without transfusions. Six postoperative deaths were attributed to anaesthesia complications. Serjeant[110] is of the opinion that major surgery, which is commonly conducted in Jamaica at steady state haemoglobin levels of 70–90 $g \cdot L^{-1}$ with only replacement of surgical blood loss, carries minimal morbidity and no mortality.

Rambo *et al.*[111] reported 11 sickle cell anaemia (SS) patients undergoing elective cholecystectomy, of whom nine were transfused preoperatively. Their recommendation was to achieve a haematocrit of 30 per cent preoperatively. The postoperative morbidity from this 1977–1984 series was limited to one case of pneumonia in a transfused patient. This was a better outcome than reported in an earlier series from the same South Carolina institution.[112] In the 16 surgical patients treated from 1968–1977, there was a morbidity of 37 per cent including one death. Rutledge[113] reports on 42 patients for elective cholecystectomy who were transfused preoperatively. Postoperatively, 16.7 per cent had complications and one 16-year-old boy probably died from pulmonary infarction. They concluded that cholecystectomy is associated with a higher complication rate in sickle cell anaemia patients than normal patients.

Lagarde and Tunell[114] described their findings in 65 children with sickle cell anaemia and three with sickle cell trait who underwent 115 operations, involving major surgery in 50 per cent. Details of the transfusion therapy are not given but 37.4 per cent of the patients received transfusions at some time during the procedures. They

TABLE II Guidelines for preoperative transfusion preparation for surgery (Montreal Children's Hospital Programme)

Haemoglobin status	*Procedure*	*Transfusion*	*Aims*
Sickle cell trait (AS) (normal Hb)	All surgery	Not usually indicated	
Sickle cell anaemia SS type (low Hb)	Minor surgery short duration	Simple transfusion	Hct = 30–36%
	*Major surgery long duration	Partial exchange transfusion	Hct = 30–36% HbS ≤ 30%
Sickle cell anaemia SC type and SB thalassemia	All procedures	Partial exchange transfusion	Hct = 30–36% HbS ≤ 30%

*Includes cholecystectomy, cardiac, neurosurgical and intraocular procedures.

recommended preoperative transfusion to a haematocrit of 38–40 per cent. There was one unexplained death two days post-tonsillectomy and 21 patients had postoperative infections. Coker and Milner[115] in 1982 reported nine children undergoing tonsillectomies, all transfused to reduce the HbS below 45 per cent preoperatively, who showed no complications.

Janik and Seeler,[88] in Chicago, reported that 35 children with sickle cell anaemia who underwent 46 operations from 1967 to 1978 had no complications. All had been prepared preoperatively by simple transfusion to achieve a haematocrit of 36 per cent. In their opinion, the preoperative transfusion resulted in the freedom from morbidity and mortality. However. Spigelman *et al.*,[116] in Los Angeles had earlier reported on 12 paediatric surgical patients undergoing 29 operations from 1962 to 1971. Blood transfusion was advocated at that time and seven simple and four exchange transfusions were given preoperatively, two transfusions intraoperatively and five postoperatively. Despite this preparation, seven patients showed postoperative complications which included pneumonia, wound infections, and bacteraemia.

Reading these reports chronologically seems to support the view that postoperative complications are decreasing in these patients, but the reasons for this are not clear. Preoperative transfusion has become more routine practice recently but introduces the risks of transmission of serious infections, alloimmunization, iron overload and transfusion reactions. Techniques of anaesthesia and patient monitoring have also improved to the point where death attributable to anaesthesia has been reduced to one in 10,000 anaesthetics.[117] It remains difficult to separate the role of transfusion therapy from these other factors in the improvements in outcome for patients with sickle cell states.

A controversial aspect of transfusion therapy is the level of HbS which is considered safe. Arbitrary recommendations vary from HbS of 40 per cent for general surgery to less that five per cent for cardiopulmonary bypass with hypothermia.[118] However, adults may refuse transfusion because of their religious beliefs or concerns for the risks associated with transfusion. In paediatric anaesthesia, recommendations for treatment which are contrary to those of the parents may be difficult to impose. Resulting patterns of practice vary and the outcomes may be obscured because of the heterogeneous nature of sickle cell disease. This heterogeneity may be relevant to which patients benefit from blood transfusions and for which types of surgery it is important. Recommendations for preoperative transfusion practices are constantly undergoing review, but our current practice is summarized in Table II.

The obstetric patient

The outcome of pregnancy in parturients with sickle cell trait should be unaltered. The death of a sickle cell trait patient during Caesarian section due to aorto-caval compression, despite lateral tilting, has recently been reported.[104] Those with SS, SC or S beta-thalassemia disease have been thought to be poor obstetric risks. Anaemia becomes more severe during pregnancy and there is an increased risk of abortion, stillbirth, toxaemia and urinary tract infections.[119,120] While the incidence of these complications may have decreased due to improvements in general prenatal care and the use of simple or exchange transfusions to control the disease process, there has been a concomitant increased survival and pregnancy rate in high-risk patients in recent years.

Geographical variations in reporting the incidence of maternal and fetal complications are also apparent. However, a study of pregnancies in homozygous sickle cell disease patients in Jamaica from 1959 to 1984[121]

showed that 71.5 per cent had a good outcome. Maternal mortality was 1.1 per cent with deaths occurring in late pregnancy or one to five days post partum due to pulmonary emboli, peritoneal haemorrhage or pneumonia. Fetal wastage resulted from spontaneous abortion in 11 per cent and stillbirths in 10.5 per cent. Prematurity and low birth weight are factors in perinatal mortality, possibly because of sickling in the uterine blood vessels and placental infarction. The Caesarian section rate of 24 per cent in the SS patients was twice that in other obstetric patients.

The efficacy of prophylactic blood transfusion in altering the fetal or maternal outcome has not been clarified. A retrospective study from the U.K.[122] showed no differences between those patients receiving prophylaxis and those who were not transfused. However, the use of transfusion was associated with immediate transfusion reactions in 14 per cent of patients and the formation of red cell antibodies in a further 22 per cent.

The choice of regional or general anaesthesia for obstetric patients depends on the patient's general condition and the preference of the anaesthetist, rather than specific indications. The relative risks of hypotension and hypoxia using either approach must be assessed in individual cases.

Neonates and infants

Children affected with sickle cell disease at birth have a major part of their haemoglobin as haemoglobin F and may not be anaemic. The homozygous SS patient has less than 20 per cent HbS. This situation is generally protective against vasoocclusive events. Symptomatic disease can be a problem in this age group infrequently[10,123] but no special precautions should be needed for general anaesthesia in the neonate.

The child at risk should be screened in the newborn period to facilitate entry into comprehensive care programmes. All children with sickle cell disease should start prophylactic penicillin at age three months.[62] Splenic function is compromised early in life in these children.[21] In infancy the major causes of death are infection and acute splenic sequestration.[11]

The patient for open heart surgery

The coexistence of sickle cell disease or trait in patients undergoing open heart surgery (OHS) has been infrequent. However, cardiac surgery is now developing in countries where sickle cell disease is prevalent.[124,125] Also, the indications for corrective cardiac surgery at all ages are increasing. Congenital anomalies, previously considered to be inoperable, are now amenable to correction in the early neonatal period. Rheumatic and syphilitic valvular heart disease are declining but the indications for surgery requiring CPB in adults have increased with the popularity of coronary artery bypass grafting. Thus, the occurrence of dual pathology, necessitating OHS in sickle cell disease and trait patients, is expected to become more common.

Reports of the successful management of these patients are limited, but include more children with homozygous or doubly heterozygous sickle cell anaemia[126–130] than adults.[124–126,132] An early report of a death during aortic valve replacement[133] due to sickling intraoperatively drew attention to the potential risk of CPB in patients with sickle cell trait. However, the patient was never diagnosed by electrophoresis and the autopsy findings were not conclusive. Subsequently, results in this group have been favourable in both children[134–136] and adults.[125,134,137–140] Few perioperative complications have been reported and the role of the haemoglobinopathy in their aetiology is not clear. Sivapragasam,[125] with the largest series of 30 sickle cell trait patients, reports eight deaths within four weeks of surgery: one patient died at induction of anaesthesia while the others showed evidence of recent infarctions in kidneys, lungs, brain, liver and myocardium at autopsy. However, this operative mortality of 26 per cent was close to the 23 per cent mortality found in similar surgical patients without haemoglobin S. Complications treated in the immediate postoperative period in 18 of these patients (60 per cent) included cardiac, respiratory and renal failures, conduction defects and cardiac arrythmias, haematuria and hemorrhage.

Management of CPB for patients with sickle cell trait and disease varied widely with respect to duration of circulatory arrest and core temperature during CPB [utilizing normothermia,[124,137] moderate hypothermia[123,124,134] or profound hypothermia].[135,138,139] There was no consensus on the role of blood transfusion in management, although measurements of the percentage of HbS preoperatively, during CPB, and postoperatively were available in most instances. Partial exchange transfusion[127,130,138,139] or simple transfusion[124,125,128,139] was used preoperatively in some cases. In others, blood was utilized intraoperatively as a constituent of the priming solution[123,128,129,135,139] as a simple transfusion,[132,133,139] or as a partial exchange transfusion.[129,131,134,136,137]

These experiences showed an awareness of the risks of undertaking CPB on patients with HbS, but failed to provide clear guidelines for management. Recommendations which can be made must be based on theoretical considerations of the nature of the sickle haemoglobinopathy. Cardiopulmonary bypass imposes those extreme physiological insults which should be avoided in patients with HbS. These result from low-flow states, circulatory

arrest, or aortic cross-clamping with hypoxia and acidosis, total body hypothermia with lowering of core temperature with or without topical cooling and the infusion of cold cardioplegia, and possibly mechanical haemolysis of red cells by the pump or prosthetic heart valves. Sickling occurs in SS patients at oxygen tensions below 5.3 kpa (40 mmHg) to 6.0 kPa (45 mmHg)[124] and in AS patients at 2.7 kPa (20 mmHg) to 3.3 kPa (25 mmHg). However, such hypoxic conditions are unusual during CPB, except during conditions of low flow and total circulatory arrest.

However, *in vitro* studies have shown that hypothermia may be protective against sickling in that the rate of gel formation is reduced as temperature is reduced. It is the associated vasoconstriction and vascular stasis with increasing capillary transit time that appears to be the determinant of the onset of sickling.[38] If care is taken to ensure adequate peripheral perfusion and oxygenation by administration of a vasodilator and lowering of blood viscosity, the beneficial effects of hypothermia in preventing sickling will predominate.[136] No recommendations can be made at present on safe levels of hypothermia during CPB.

Blood transfusion is the only therapeutic adjunct which can reduce the percentage of HbS and lower the population of red cells available for sickling. Lowering the concentration of HbS by simple transfusion or haemodilution using a blood or crystalloid prime will achieve this. Although this will lessen the percentage of circulating cells containing HbS, those present will still be at risk for sickling under the appropriate conditions, but the consequences will be less severe. Partial exchange transfusion is necessary to remove HbS cells from the circulation when the percentage is very high initially or more complete removal as necessary. Goals for the lowering of HbS to 30 per cent in the presence of a haematocrit of 36 per cent preoperatively have been established for major surgery. While this may also be acceptable before CPB, recommendations for more complete removal are common[136,140] and in children it is suggested that HbS below five per cent is desirable.[118] A safe level of HbS for these patients has not yet been firmly established.

The timing of transfusion therapy is also controversial. Patients with cardiac disease may be chronically hypoxaemic and show severe symptoms of sickle cell anaemia. Separation of symptoms of cardiac disease from those of sickle cell anaemia may be difficult, as cardiac murmurs, joint pains, dyspneoa and cor pulmonale may occur as a result of either. However, the risk to the patient in cardiac failure of simple or partial exchange transfusion causing fluid overload, pulmonary oedema and low output states, must be balanced by the potential benefit to a patient in sickle cell crisis. Preoperative partial exchange transfusion to lower HbS to 40 per cent is common[131] but not universal practice.[130,134] Although, for safety during anaesthesia induction and early surgery before commencing CPB, it would seem to be indicated.

There is a dramatic fall in the percentage of HbS once CPB is instituted, whether a crystalloid or blood-containing priming solution is used. Initial dilution reduces the amount by about one-third and a further similar reduction may occur during CPB. Postoperatively, lower levels remain for at least two days before gradually regaining preoperative levels.[130] Thus, if the early operative period has passed, CPB can be expected to ameliorate the condition. In patients in whom blood is withheld preoperatively, this can be anticipated. No attention has been given to the crystalloid constituents of the prime, but the avoidance of hyperosmolality is important.

The potential hazards to sickle cell trait and disease patients of OHS may have been underestimated. Certainly the conditions prevail for intravascular sickling and recommendations for optimal management, as suggested in this discussion, are extremely tentative.

Acknowledgements

We are grateful to Patricia Sabatini, Esther Nuguid and Jennifer Morrison for photomicrographs and illustrations, to the secretarial assistance of Miss Lucia Lam Ham, Miss Joanne Zabihaylo and Mrs. Leonie Lafontaine.

Appendix I

Standard laboratory methods used in the diagnosis of sickle cell anaemia

Sickleprep and Sickledex

The Sickleprep and Sickledex (Ortho) are tests that should only be confirmatory after cellulose acetate electrophoresis is done. These tests are not screening tests and are not useful in precise haemoglobin identification. The Sickledex reaction is based on the relative insolubility of HbS. The Sickledex reagent powder is added to a blood sample with the Sickledex test solution. Solutions of HbS form a cloudy turbid suspension. False positives derive from hypergammaglobulinaemia or other high proteins and false negatives occur with low concentrations of HbS below ten per cent (such as in the newborn).

An older test, the sodium metabisulphite sickle cell preparation looks directly at red cells containing sickle haemoglobin. A drop of fresh two per cent sodium metabisulphite is mixed on a slide with a drop of blood and the cover slip applied and sealed with Vaseline. This hypoxic environment causes sickling of HbS containing cells. The preparation is examined with the high-power dry objective under low light. One thousand cells are

counted and the percentage of sickled cells is obtained. Certain poikilocytes can be difficult to distinguish from sickled cells, severe iron deficiency can interfere with sickling and cord blood has such a low percentage of HbS (as few as two per cent can be sickled) that the sickled cells are missed.

These tests have the advantage of being relatively cheap, technically easy and can be done on finger prick samples of blood. The disadvantages are that they cannot distinguish sickle trait from disease and are not useful in the newborn period because of the the high proportion of HbF which is present. They give no information about other haemoglobins besides HbS and can be falsely negative in young children with SC type sickle cell aenemia. They should only be used to confirm the presence of sickle haemoglobin in older children. Haemoglobin electrophoresis is the test of choice for accurate diagnosis.

Haemoglobin electrophoresis on cellulose acetate

Cellulose acetate haemoglobin electrophoresis is the standard for haemoglobin separation and is the method of choice in screening for sickle cell disease. Abnormal bands found by this method must be further studied using citrate agar electrophoresis and the presence of HbS confirmed by the sickle cell prep. Cellulose acetate is also relatively cheap and easy and available in kit form. Haemolysates are applied to a cellulose acetate medium with a buffer of pH 8.4–9.2, under constant voltage direct current power supply up to 450 V, for about 20 minutes. Ponceau S staining is used. HbA is negatively charged in an alkaline buffer and moves toward the anode. Most haemoglobin variants separate from HbA because their structural abnormality changes the electrical charge. The haemoglobins in the test blood sample migrate into distinct bands.

Haemoglobin electrophoresis on citrate agar

Citrate agar electrophoresis is used to confirm results found on cellulose acetate. 1.5% agar is the support medium. Haemoglobins separate by charge but also by solubility or their adsorption to the medium. This method is used every time an abnormal band is found by cellulose acetate. It will confirm the presence of HbS, A, F and C. It will also differentiate HbC from HbE and O and will distinguish HbS from other variants such as HbG and D.

Bibliography

Huntsman RG. Sickling tests – microscopic and non-microscopic in the detection of haemoglobinopathies. Schmidt RM, Huisman THJ, H. Lehman (Eds). 1974, CRC Press, Cleveland, Ohio, pp. 15–9.

National Committee for Clinical Laboratory Standards. Detection of abnormal haemoglobins using cellulose acetate electrophoresis; approved standard. NCCLS publication H8-A. Villanova, PA: NCCLS, 1986.

Laboratory methods for detecting haemoglobinopathies. Centers for Disease Control, Public Health Service, US Department of Health and Human Services, Atlanta, Georgia, September 1984.

NIH Consensus Development Conference. Newborn screening for sickle cell disease and other haemoglobinopathies. April 6–8, 1987, Washington, D.C.

Galacteros F, Kleman K, Caburi-Martin J. Beauzard Y, Rosa J, Lubin B. Cord blood screening for haemoglobin abnormalities by thin layer isoelectric focusing. Blood 1980; 56: 1068–71.

Appendix II

Protocol for the management of the patient with sickle cell anaemia

Preoperative guidelines of the Montreal Children's Hospital

1 All patients from ethnic groups at risk (primarily patients with Caribbean and African ancestry) for sickle cell disease should be screened by haemoglobin electrophoresis (not Sickleprep or Sickledex).
2 Confirm the haemoglobin diagnosis by the haemoglobin electrophoresis. Distinguish trait from disease and specify exact type of haemoglobinopathy. Indicate whether Hb SS, Hb SC or Hb S thalassaemia. This can only be done by the Haematology Consultant. The sickledex or sickleprep is not useful as a single test and is never useful in young children.
3 Establish baseline CBC with reticulocyte count, hepatitis B antigen status and HIV status if risk factors present. Crossmatch to include an extended red cell phenotype.
4 Record a thorough medical history and complete physical examination. Note carefully the presence of infection and any relevant organ dysfunction (CNS, pulmonary, renal, cardiac, etc.). An accurate transfusion history is essential.
5 Institute folic acid supplement. (Dose: 5 mg orally daily or weekly.)
6 Ensure anti-infection precautions:
   (a) Pneumococcal and haemophilus influenza vaccinations are required for children 2 years of age or older who have not received them previously.
   (b) Prophylactic daily penicillin (Dose: 125 mg bid under three years and 250 mg bid over three years)

is required from age three months until seven years old.

(c) Depending on the surgical procedure, consider prophylactic antibiotics. Coverage is usually recommended for many dental procedures.

(d) Ensure close postoperative monitoring for infectious complications, especially pulmonary. Aim for early mobilization.

7 In cooperation with the haematology consultant determine the need for:

(a) Simple transfusion to achieve a Hb of 90–100 $g \cdot L^{-1}$. This procedure may be satisfactory for short duration procedures in very anaemic patients.

(b) Partial exchange tranfusion to specifically decrease the percentage of HbS cells to 30 per cent. Most major operations require this procedure.
It should be noted that this is best done electively over two to three days with sufficient time allowed to order the correct amount and type of blood. The course of the exchange can be quantitated by the laboratory using the one hour prep during emergency hours or the densitometer during laboratory hours.

8 Ask the blood bank for irradiated blood products and phenotypically matched red cells. The latter requires a crossmatch in advance of surgery where "red blood cell phenotype" is written on the requisition in addition to the blood grouping and number of units required.

9 *Special cases*

CHILDREN LESS THAN SIX MONTHS OF AGE:

Sickle cell anaemia can be diagnosed in the newborn period so that *all* children regardless of age should be screened by the two methods of haemoglobin electrophoresis (cellulose acetate and citrate agar). The Sickleprep and Sickledex are *not* useful. Patients in this age group have sufficient Hb F so that their amount of Hb S may be low enough that exchange is not necessary. The diagnosis as early in life as possible is important because these babies need prohylactic penicillin to prevent overwhelming sepsis, an important cause of mortality in patients less than three years old. The post-operative course should be closely monitored for infectious complications. The patient needs referral to a comprehensive sickle cell program as soon as discharged. Ideally counselling and support will be started for the family while the infant is in hospital.

EMERGENCY SURGERY THAT REQUIRES GENERAL ANAESTHESIA:

There is no change from the prior protocol unless surgery occurs after 17:00 h and before 9:00 h when the haemoglobin electrophoresis laboratory is closed. In off hours, the haematologist-on-call will help with the precise diagnosis based on the relevant history, physical examination, CBC and reticulocyte count, peripheral blood smear and sickleprep. The decision on the transfusion preparation of the patient will rest with the haematologist and the anaesthetist depending on the age of the patient, the haematocrit, the nature of the surgery and the availability of blood. If the diagnosis is not clear, surgery will be delayed if possible.

References

1 *Miller DR, Baehner RL, McMillan CW.* (Eds). Blood Diseases of Infancy and Childhood. 5th ed. Toronto: CV Mosby, 1984 p. 406.

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

3 *Scriver JB, Waugh TR.* Studies on a case of sickle-cell anemia. Can Med Assoc J 1930; 23: 375–80.

4 *Kan YW, Dozy AM.* Evolution of the hemoglobin S and C genes in world populations. Science 1980; 209: 388–91.

5 *Antonarakis SE, Boehm CD, Serjeant GR, Thiesen CE, Dover GJ, Kazazian HH.* Origin of the β^S-globin gene in Blacks: the contribution of recurrent mutation or gene conversion or both. Proc Natl Acad Sci USA 1984; 81: 853–6.

6 *Serjeant GR.* Sickle Cell Disease. New York: Oxford University Press, 1985 pp. 14–23.

7 *Allison AC.* Protection afforded by sickle-cell trait against subtertian malarial infection. Br Med J 1954; 1: 290–4.

8 *Kark JA, Posey DM, Schumacher HR, Ruehle CJ.* Sickle-cell trait as a risk factor for sudden death in physical training. N Engl J Med 1987; 317: 781–7.

9 *Sullivan LW.* The risks of sickle-cell trait: caution and common sense. N Engl J Med 1987; 317: 830–1.

10 *Karayalcin G.* Sickle cell anemia in the neonatal period. South Med J 1979; 72: 492–3.

11 *O'Brien RT, McIntosh S, Aspnes GT, Pearson HA.* Prospective study of sickle cell anemia in infancy. J Pediatr 1976; 89: 205–10.

12 *Rao S, Gooden S.* Splenic sequestration in sickle cell disease: role of transfusion therapy. Am J Pediatr Hematol/Oncol 1985; 7: 298–301.

13 *Powars D, Wilson B, Imbus C, Pegelow C, Allen J.* The natural history of stroke in sickle cell disease. Am J Med 1978; 65: 461–71.

14 *Russell MO, Goldberg HI, Hodson A et al.* Effect of transfusion therapy on arteriographic abnormalities and on recurrence of stroke in sickle cell disease. Blood 1984; 63: 162–9.

15 *Cohen SB, Fletcher ME, Goldberg MF, Jednock NJ.* Diagnosis and management of ocular complications of

sickle hemoglobinopathies (5 parts). Opthalmic Surgery 1986; 17: 57–9, 110–6, 184–8, 312–5, 369–74.

16 *Sarnaik SA, Slovis TL, Corbett DP, Emami A, Whitten CF.* Incidence of cholelithiasis in sickle cell anemia using the ultrasonic gray-scale technique. J Pediatr 1980; 96: 1005–8.

17 *Tarry WF, Duckett JW Jr, Snyder H McC.* Urological complications of sickle cell disease in a pediatric population. J Urol 1987; 138: 592–4.

18 *Serjeant GR.* Leg ulceration in sickle cell anemia. Arch Int Med 1974; 133: 690–4.

19 *Hawker H, Neilson H, Hayes RJ, Serjeant GR.* Haematological factors associated with avascular necrosis of the femoral head in homozygous sickle cell disease. Br J Haematol 1982; 50: 29–34.

20 *Poncz M, Kane E, Gill FM.* Acute chest syndrome in sickle cell disease: etiology and clinical correlates. J Pediatr 1985; 107: 861–6.

21 *Pearson HA, Gallagher D, Chilcote R et al.* (The Cooperative Study of Sickle Cell Disease.) Developmental pattern of splenic dysfunction in sickle cell disorders. Pediatrics 1985; 76: 392–7.

22 *Serjeant GR, Mason K, Topley JM, Serjeant BE.* Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. Lancet 1981; 2: 595–7.

23 *Bunn HF, Forget BG.* Hemoglobin: Molecular, Genetic and Clinical Aspects. Toronto: WB Saunders, 1986, p. 512.

24 *Emond AM, Venugopal S, Morais P, Carpenter RG, Serjeant GR.* Role of splenectomy in homozygous sickle cell disease in childhood. Lancet 1984; 1: 88–91.

25 *Embury SH, Dozy AM, Miller J et al.* Concurrent sickle-cell anemia and a-thalassemia. Effect on severity of anemia. N Engl J Med 1982; 306: 270–4.

26 *Schechter AN, Bunn HF.* What determines severity in sickle-cell disease? N Engl J Med 1982; 306: 295–7.

27 *Miller BA, Olivieri N, Salameh M et al.* Molecular analysis of the High-hemoglobin-F phenotype in Saudi Arabian sickle cell anemia. N Engl J Med 1987; 316: 244–50.

28 *Pauling L, Itano HA, Singer SJ, Wells IC.* Sickle cell anemia, a molecular disease. Science 1949; 110: 543–8.

29 *Ingram VM.* Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin. Nature 1957; 180: 326–8.

30 *McCurdy PR, Sherman AS.* Irreversibly sickled cells and red cell survival in sickle cell anemia. A study with both DF32P and 51Cr. Am J Med 1978; 64: 253–8.

31 *Evans E, Mohandas N, Leung A.* Static and dynamic rigidities of normal and sickle erythrocytes: major influence of cell hemoglobin concentration. J Clin Invest 1984; 73: 116–23.

32 *Mohandas N, Evans E.* Sickle cell adherence to vascular endothelium: morphologic correlates and the requirement for divalent cations and collogen-binding plasma proteins. J Clin Invest 1985; 76: 1605–12.

33 *Hebbel RP.* Auto-oxidation and a membrane-associated "Fenton Reagent": a possible explanation for development of membrane lesions in sickle erythrocytes. Clin Haematol 1985; 14: 129–40.

34 *Noguchi CT, Dover GJ, Rodgers GP et al.* Alpha-thalassemia changes erythrocyte heterogeneity in sickle cell disease. J Clin Invest 1985; 75: 1632–37.

35 *Sherwood JB, Goldwasser E, Chilcote R et al.* Sickle cell anemia patients have low erythropoietin levels for their degree of anemia. Blood 1986; 67: 46–9.

36 *Murayama M.* Molecular mechanism of red cell "sickling." Science 1966; 153: 145–9.

37 *Dykes GW, Crepeau RH, Edelstein SJ.* Three-dimensional reconstruction of the 14-filament fibers of hemoglobin S. J Mol Biol 1979; 130: 451–72.

38 *Eaton WA, Hofrichter J.* Hemoglobin S gelation and sickle cell disease. Blood 1987; 70: 1245–66.

39 *Nagel RL, Bookchin RM, Johnson J et al.* Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S. Proc Natl Acad Sci USA 1979; 76: 670–2.

40 *Singer K, Singer L.* The gelling phenomenon of sickle cell hemoglobin; its biological and diagnostic significance. Blood 1953; 8: 1008–23.

41 *Eaton WA, Hofrichter J, Ross PD.* Delay time of gelation; a possible determinant of clinical severity in sickle cell disease. Blood 1976; 47: 621–7.

42 *Hofrichter J, Ross PD, Eaton WA.* Kinetics and mechanism of deoxyhemoglobin S gelation: a new approach to understanding sickle cell disease. Proc Natl Acad Sci USA 1974; 71: 4864–8.

43 *Seakins M, Gibbs WN, Milner PF, Bertles JF.* Erythrocyte HbS concentration. An important factor in the low oxygen affinity of blood in sickle cell anemia. J Clin Invest 1973; 52: 422–32.

44 *Bromberg PA, Jensen WN.* Blood oxygen dissociation curves in sickle cell disease. J Lab Clin Med 1967; 70: 480–7.

45 *Noguchi CT, Torchia DA, Schechter AN.* Intracellular polymerization of sickle hemoglobin: effects of cell heterogeneity. J Clin Invest 1983; 72: 846–52.

46 *Briehl RW.* The effects of shear on the delay time for gelation of hemoglobin S. Blood Cells 1982; 8: 201–12.

47 *Bookchin RM, Lew VL.* Red cell membrane abnormalities in sickle cell anemia. Prog Hematol 1983; 13: 1–23.

48 *Bertles JF, Milner PFA.* Irreversibly sickled erythrocytes: a consequence of the heterogeneous distribution

of hemoglobin types in sickle cell anemia. J Clin Invest 1968; 47: 1731–41.

49 *Nash GB, Johnson CS, Meiselman HJ.* Mechanical properties of oxygenated red blood cells in sickle cell (Hb SS) disease. Blood 1984; 63: 73–82.

50 *Lubin B, Chiu D, Bastacky J, Roelofsen B, van Deenen LLM.* Abnormalities in membrane phospholipid organization in sickled erythrocytes. J Clin Invest 1981; 67: 1643–9.

51 *Rank B, Carlsson J, Hebbel RP.* Abnormal redox status of membrane-protein thiols in sickle erythrocytes. J Clin Invest 1985; 75: 1531–7.

52 *Chiu D, Lubin B, Roelofsen B, van Deenen LLM.* Sickled erythrocytes accelerate clotting in vitro: an effect of abnormal membrane lipid asymmetry. Blood 1981; 58: 398–401.

53 *Rodgers GP, Schechter AN, Noguchi CT, Klein HG, Nienhuis AW, Bonner RF.* Periodic microcirculatory flow in patients with sickle cell disease. N Engl J Med 1984; 311: 1534–8.

54 *Chang JC, Kan YW.* Antenatal diagnosis of sickle cell anemia by direct analysis of the sickle mutation. Lancet 1981; 2: 1127–9.

55 *Conner BJ, Reyes AA, Morin C, Itakura K, Teplitz RD, Wallace RB.* Detection of sickle cell Bs-globin allele by hybridization with synthetic oligonucleotides. Proc Natl Acad Sci USA 1983; 80: 278–82.

56 *Kan YW, Dozy AM.* Polymorphism of DNA sequence adjacent to human B-globin structural gene: relationship to sickle mutation. Proc Natl Acad Sci USA 1978; 75: 5631–5.

57 *Wainscoat JS, Bell JI, Thein SL et al.* Multiple origins of the sickle mutation: evidence from Bs-globin gene cluster polymorphisms. Mol Biol Med 1983; 1: 191–7.

58 *Mears JG, Beldjord C, Benabadji M, Belghiti YA, Baddou M, Labie D, Nagel RL.* The sickle gene polymorphism in North Africa. Blood 1981; 58: 599–601.

59 *Mears JG, Lachman HM, Cabannes R, Amegnizin KPE, Labie D, Nagel RL.* Sickle gene: its origin and diffusion from West Africa. J Clin Invest 1981; 68: 606–10.

60 *Headings VE.* Sickle cell disease in childhood. Strategies for early diagnosis. Am J Pediatr Hematol/Oncol 1982; 4: 67–71.

61 *Yawson GI, Huntsman RG, Metters JS.* An assessment of techniques suitable for the diagnosis of sickle-cell disease and haemoglobin C disease in cord blood samples. J Clin Path 1970; 23: 533–7.

62 *Gaston MH, Verter JI, Woods G et al.* For the prophylactic penicillin study group. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. N Engl J Med 1986; 314: 1593–9.

63 *Chang JC, Kan YW.* A sensitive new prenatal test for sickle-cell anemia. N Engl J Med 1982; 307: 30–2.

64 *Goosens M, Dumez Y, Kaplan L et al.* Prenatal diagnosis of sickle-cell anemia in the first trimester of pregnancy. N Engl J Med 1983; 309: 831–3.

65 *Rabb LM, Grandison Y, Mason K, Hayes RJ, Serjeant BE, Serjeant GR.* A trial of folate supplementation in children with homozygous sickle cell disease. Br J Haematol 1983; 54: 589–94.

66 *Weintrub PS, Schiffman G, Addiego JE et al.* Long-term follow-up and booster immunization with polyvalent pneumococcal polysaccharide in patients with sickle cell anemia. J Pediatr 1984; 105: 261–3.

67 *Peltola H, Kayhty H, Virtanen M, Makela PH.* Prevention of hemophilus influenzae type B bacteremic infections with the capsular polysaccharide vaccine. N Engl J Med 1984; 310: 1561–6.

68 *Aluoch JR.* The treatment of sickle cell disease. A historical and chronological literature review of the therapies applied since 1910. Trop Georgr Med 1984; 36: S1–S26.

69 *Charache S, Walker WG.* Failure of desmopressin to lower serum sodium or prevent crisis in patients with sickle cell anemia. Blood 1981; 58: 892–6.

70 *Rosa RM, Bierer BE, Bunn HF, Epstein FH.* The treatment of sickle cell anemia with induced hyponatremia. Blood Cells 1982; 8: 329–35.

71 *Schoomaker EB, Brewer GJ, Oelshlegel FJ.* Zinc in the treatment of homozygous sickle cell anemia: studies in an animal model. Am J Hematol 1976; 1: 45–7.

72 *Berkowitz LR, Orringer EP.* Effects of citicdil on monovalent cation permeability in the erythrocyte: an explanation for the efficacy of citiedil in the treatment of sickle cell anemia. Blood Cells 1982; 8: 283–8.

73 *Embury SH, Garcia JF, Mohandas N, Pennathur-Das R, Clark MR.* Effects of oxygen inhalation on endogenous erythropoietin kinetics, erythropoiesis and properties of blood cells in sickle-cell anemia. N Engl J Med 1984; 311: 291–5.

74 *Charache S, Dover GJ, Moyer MA, Moore JW.* Hydroxyurea-induced augmentation of fetal hemoglobin production in patients with sickle cell anemia. Blood 1987; 69: 109–16.

75 *Veith R, Galanello R, Papayannopoulou Stamatoyannopoulos G.* Stimulation of F-cell production in patients with sickle cell anemia treated with cytarabine or hydroxyurea. N Engl J Med 1985; 313: 1571–5.

76 *Platt OS.* Chemotherapy to increase fetal hemoglobinin patients with sickle cell anemia. Am J Pediatr Hematol/Oncol 1985; 7(3): 258–60.

77 *Johnson FL, Look AT, Gockerman J, Ruggiero MR, Dalla-Pozza Luciano, Billings FT.* Bone-marrow transplantation in a patient with sickle-cell anemia. N Engl J Med 1984; 311: 780–3.

78 *Shapiro LJ, Comings DE, Jones OW, Rimoin DL.* New

frontiers in genetic medicine. Ann Int Med 1986; 104: 527–39.

79 *Dover GJ, Chang VT, Boyer SH, Serjeant GR et al.* The cellular basis for different fetal hemoglobin levels among sickle cell individuals with two, three, and four alpha-globin genes. Blood 1987; 69: 341–4.

80 *Ley TJ, DeSimone J, Noguchi CT et al.* 5-azacytidine increases gamma-globin synthesis and reduces the proportion of dense cells in patients with sickle cell anemia. Blood 1983; 62: 370–80.

81 *Johnson FL.* Bone marrow transplantation in the treatment of sickle cell anemia. Am J Pediatr Hematol/Oncology 1985; 7: 254–7.

82 *Charache S, Lubin B, Reid CD (Eds).* Management and Therapy of Sickle Cell Disease. US Dept. of Health and Human Services, Public Health Service, National Institutes of Health. NIH Publication no. 85-2117, September 1985.

83 *Lanzkowsky P, Shende A, Karayalcin G, Kim Y-J, Aballi AJ.* Partial exchange transfusion in sickle cell anemia. Am J Dis Child 1978; 132: 1206–8.

84 *Chernoff AI, Shapleigh JB, Moore CV.* Therapy of chronic ulceration of the legs associated with sickle cell anemia. JAMA 1954; 155: 1487–91.

85 *Charache S, Scott J, Niebyl J, Bonds D.* Management of sickle cell disease in pregnant patients. Obstetr Gynecol 1980; 55: 407–10.

86 *Schmalzer EA, Lee JO, Brown AK, Usami S, Chien S.* Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels. Implications for transfusion. Transfusion 1987; 27: 228–33.

87 *Laasberg LH, Hedley-Whyte J.* Viscosity of sickle disease and trait blood: changes with anesthesia. J Appl Physiol 1973; 6: 837-43.

88 *Janik J, Seeler RA.* Perioperative management of children with sickle hemoglobinopathy. J Ped Surg 1980; 15: 117–20.

89 *Davies SC, McWilliam AC, Hewitt PE, Devenish A, Brozovic M.* Red cell alloimmunization in sickle cell disease. Br J Haematol 1986; 63: 241–5.

90 *Vichinsky E, Giardot C, Johnson R et al.* Alloimmunization in sickle cell disease (Abstract). Blood 1987; 70: 335a.

91 *Cohen A, Schwartz E.* Iron chelation therapy in sickle cell anemia. Am J Hematol 1979; 7: 69–76.

92 *Milner PF, Squires JE, Larison PJ, Charles WT, Krauss JS.* Post transfusion crises in sickle cell anemia: role of delayed hemolytic reactions to transfusion. Southern Med J 1985; 78: 1462–9.

93 *Scott RB.* Historical review of legislative and national initiatives for sickle cell disease. Am J Pediatr Hematol/Oncol 1983; 5: 346–51.

94 *Gaston M, Rosse WF.* The cooperative study of sickle cell disease. Review of study design and objectives. Am J Pediatr Hematol/Oncol 1982; 4: 197–201.

95 *Huntsman RG.* Sickle-cell Anemia and Thalassemia: a Primer for Health Care Professionals. Toronto, Canadian Sickle Cell Society, 1987.

96 *Oduro KA.* Anaesthesia in Ghana: a review with particular reference to the indigenous medical conditions. Anaesthesia 1969; 24: 307–16.

97 *Oduntan SA, Isaacs WA.* Anaesthesia in patients with abnormal haemoglobin syndromes: a preliminary report. Br J Anaesth 1971; 43: 1159–66.

98 *Howells TH, Huntsman RG, Boys JE, Mahmood A.* Anaesthesia and sickle-cell haemoglobin: with a case report. Br J Anaesth 1972; 44: 975–87.

99 *Searle JF.* Anaesthesia in sickle cell states: a review. Anaesthesia 1973; 28: 48–58.

100 *Burrington JD, Smith MD.* Elective and emergency surgery in children with sickle cell disease. Surg Clin N Amer 1976; 56: 55–71.

101 *Weingarten A.* Anesthesia and the patient with sickle cell anemia. Clin Anesthesiology 1985; 3: 1–8.

102 *Luban NLC, Epstein BS, Watson SP.* Sickle cell disease and anesthesia. *In*: Advances in Anesthesia, Gallagher TJ (Ed). Chicago: Year Book Med Pub. 1984, pp. 289–336.

103 *Kaufman L, Sumner E.* Medical problems and the anaesthetist. London. Edward Arnold. 1979. pp. 139–51.

104 The Anaesthesia Advisory Committee to the Chief Coroner of Toronto. Intraoperative death during Caesarian section in a patient with sickle-cell trait. Can J Anaesth 1987; 34: 67–70.

105 *Brustowicz RM, Moncorge C, Koka BV.* Metabolic responses to tourniquet release in children. Anesthesiology 1987; 67: 792–4.

106 *Rosenbaum JM.* Fatal hemoglobin S-C disease crises following tonsillectomy. Arch Otolaryng 1965; 82: 307–9.

107 *Rockoff AS, Christy D, Zeldis N et al.* Myocardial necrosis following general anesthesia in hemoglobin SC disease. Pediatrics 1978; 61: 73–6.

108 *Holzmann L, Finn H, Lichtman HC, Harmel MH.* Anesthesia in patients with sickle cell disease: a review of 112 cases. Anesth Analg 1969; 48: 566–72.

109 *Homi J, Reynolds J, Skinner A, Hanna W, Serjeant G.* General anesthesia in sickle cell disease. Br Med J 1979; 1: 1599–601.

110 *Serjeant GR.* Sickle Cell Disease. New York: Oxford University Press, 1985. p. 372.

111 *Rambo WM, Reines HD.* Elective cholecystectomy for the patient with sickle cell disease and asymptomatic cholelithiasis. Am Surgeon 1986; 52: 205–7.

112 *Gibson TJ, O'dell RF, Cathcart RS, Rambo WM.* Treatment of cholelithiasis in patients with sickle cell anemia. South Med J 1979; 72: 391–2.

113 *Rutledge R, Croom RD, Davis JW, Berkowitz LR,*

Orringer EP. Cholelithiasis in sickle cell anemia; surgical considerations. South Med J 1986; 79: 28–30.

114 *Lagarde MC, Tunell WP.* Surgery in patients with hemoglobin-S disease. J Ped Surg 1978; 13: 605–7.

115 *Coker NJ, Milner PF.* Elective surgery in patients with sickle cell anemia. Arch Otolaryngal 1982; 108: 574–6.

116 *Spigelman A, Warden MJ.* Surgery in patients with sickle cell disease. Arch Surg 1972; 104: 761–4.

117 *Davies JM, Strunin L.* Anesthesia in 1984: how safe is it? Can Med Assoc J 1984; 131: 437–41.

118 *Steward DJ.* Chapter 4: Miscellaneous disorders with significant implications for anesthesia. *In*: Manual of Pediatric Anesthesia. 2nd edition. Churchill Livingstone, New York, 1985.

119 *Shnider SM, Levinson G.* Anesthesia for Obstetrics. 2nd Edition, Williams & Wilkins, Baltimore 1987: 270–1.

120 Obstetrics: Normal & problem pregnancies. Gabbe SG, Niebyl JR, Simpson JL (Eds). Churchill Livingstone, NY, 1986: 918–21.

121 *Poddar D, Maude GH, Plant MJ, Scorer H, Serjeant GR.* Pregnancy in Jamaican women with homozygous sickle cell disease. Fetal and maternal outcome. Br J Obstet Gynaecol 1986; 93: 727–32.

122 *Tuck SM, James CE, Brewster EM, Pearson TC, Studd JWW.* Prophylactic blood transfusion in maternal sickle cell syndrome. Br J Obstet Gynaecol 1987; 94: 121–5.

123 *Hegyi T, Delphin ES, Bank A, Polin RA, Blanc WA.* Sickle cell anemia in the newborn. Pediatrics 1977; 60: 213–6.

124 *Metras D, Coulibaly AO, Ouattara K, Longechaud A, Millet P, Chauvet J.* Open-heart surgery in sickle-cell haemoglobinopathies: report of 15 cases. Thorax 1982; 37: 486–91.

125 *Sivapragasam S, Shah S, Chatoorgoon A.* Open heart surgery in patients with sickle haemoglobin, 1968–1978. W Ind Med J 1980; Supplement 29: 336–41.

126 *Harris LC, Haggard ME, Travis LB.* The coexistence of sickle cell disease and congenital heart disease: a report of three cases, with repair under cardio-pulmonary by-pass in two. Pediatrics 1964; 33: 562–70.

127 *Pearson HA, Shiebler GL, Krovetz J, Bartley TD, David JK.* Sickle cell anemia associated with tetralogy of Fallot. N Engl J Med 1965; 273: 1079–83.

128 *Craenen J, Kilman J, Hosier DM, Weinberger M.* Mitral valve replacement in a child with sickle cell anemia. J Thorac Cardiovasc Surg 1972; 63: 797–9.

129 *Szentpetery S, Robertson L, Lower RR.* Complete repair of tetralogy associated with sickle cell anemia and G-6-PD deficiency. J Thorac Cardiovasc Surg 1976; 72: 276–9.

130 *Reithmuller R, Grundy EM, Radley-Smith R.* Open heart surgery in a patient with homozygous sickle cell disease. Anaesthesia 1982; 37: 324–7.

131 *Ingram CT, Floyd JB, Santora AH.* Aortic and mitral valve replacement after sickle cell crisis (Correspondence). Anesth Analg 1982; 61: 802–3.

132 *Cameron AAC, McCord CW, Bertles JF.* Successful replacement of aortic and mitral valves with ball valve prostheses in a patient with rheumatic heart disease and SC hemoglobinopathy. Am J Cardiol 1971; 27: 318–21.

133 *Leachman RD, Miller WT, Atias IM.* Sickle cell trait complicated by sickle cell thrombi after open-heart surgery. Am Heart J 1967; 74: 268–71.

134 *de Laval MR, Taswell HF, Bowie EJW, Danielson GK.* Open heart surgery in patients with inherited hemoglobinopathies, red cell dyscrasias and coagulopathies. Arch Surg 1974; 109: 618–22.

135 *Hudson I, Davidson IA, McGregor CGA.* Mitral valve replacement using cold cardioplegia in a patient with sickle cell trait. Thorax 1981; 36: 151–2.

136 *Fox MA, Abbott TR.* Hypothermic cardiopulmonary bypass in a patient with sickle-cell trait. Anaesthesia 1984; 39: 1121–3.

137 *Yacoub MH, Baron J, Et-Etr A, Kittle CF.* Aortic homograft replacement of the mitral valve in sickle cell trait. J Thorac Cardiovasc Surg 1970; 59: 568–73.

138 *Wenham P, Scott GL, Wisheart JD.* Red cell survival after aortic valve replacement with Bjork-Shiley prosthesis in presence of sickle-cell trait. Br Heart J 1978; 40: 703–4.

139 *Heiner M, Teasdale SJ, David T, Scott AA, Glynn MFX.* Aorto-coronary bypass in a patient with sickle cell trait. Can Anaesth Soc J 1979; 26: 428–34.

140 *Chun PKC, Flannery EP, Bowen TE.* Open-heart surgery in patients with hematologic disorders. Am Heart J 1983; 105: 835–42.