Abstracts from the First International Sickle Cell Anaemia Conference

The First International Conference on Sickle Cell Anaemia was held at the University of the West Indies, Kingston, Jamaica, on January 8-10, 1969. The conference was organized by members of the Departments of Medicine and Pathology of the University of the West Indies and directed by Dr. Graham Serjeant. These abstracts are of the presentations made by the participants in the conference.

THE SICKLE CELL PHENOMENON. Wallace N. Jensen.

Hemolysis in sickle cell disease has as prerequisites the intracellular polymerization of hemoglobin, biophysical changes (such as increased rigidity of the cell) and gross shape change. The changes allow cell destruction following fractures of the long, thin, filaments followed by bulk loss of hemoglobin, or by osmotic lysis of the cell, or by erythrophagocytosis of the altered cell.

Recent studies have shown that the process of transformation from sickle to disc shape induced by oxygenation, or by hypotonic media, or by decreased temperature causes fragmentation of the cell. The process of fragmentation has been observed by phase microscopic cinematography and with stereoscanning electron beam microscopy (SEBM). These studies indicate that the fragmentation of cells during the unsickling process requires an uneven intracellular distribution of hemoglobin and an uneven rate of conversion of the crystalline hemoglobin to liquid. The unusual hemoglobin distribution causes one portion of the interior surface of the cell membrane to appose and coalesce with another segment. This results in compartmentalization of the cell. Small masses of membrane-bound hemoglobin may remain attached to the cell by thin, myelin strands, or may detach from the mother cell. The repeated sickling and unsickling of a cell may cause repetitive fragmentation and proportionally greater membrane than volume losses which eventually result in the production of a spherocyte. At the sites of cell membrane loss, there often are membrane angulations or broad folds which give the cell the appearance of an acanthocyte. These studies suggest that there are multiple mechanisms responsible for the hemolysis in sickle cell disease and show that the poikilocytes (including irreversibly sickled cells and acanthocytes) which are found in the blood of patients with sickle cell disease, result from the fragmentation process which occurs with the sickle-unsickle cycle.

SICKLE CELL ANEMIA IN ZAMBIA. G. P. T. Barclay, Kitwe, Zambia and R. G. Huntsman, Lambeth Hospital, London.

Vandepitte (1954) and J. and C. Lambotte-Legrand (1955) demonstrated in Central Africa the lethal manifestations of sickle cell anemia during infancy. It has long been known that 20 per cent of the population of the Zambian Copperbelt carry the sickle cell trait (English, 1945) and therefore 1 per cent of this population should suffer from sickle cell anemia. Prior to the adoption of any treatment trials in this area it was necessary to develop a diagnostic screening program suitable for large numbers of infants and small children. In order to attract a high acceptance rate such a routine must be based on capillary sampling only.

The series reported in Table 1 consists of 1707 children under 4 years of age presenting with varied pediatric complaints at hospital or outpatient clinics. Any patients suspected of having sickle cell anemia or siblings of a known case of sickle cell anemia were excluded from the series.

1. A sample (0.2 ml.) collected in a large capillary tube was prepared as a hemolysate and subjected to paper electrophoresis using a Tris Buffer system at pH 8.9.

2. The sickling test was carried out on a
small clotted capillary sample using 2 per cent sodium metabisulphite as a reducing agent.

3. In order to eliminate confusion due to fetal hemoglobin, any specimen from a child under 6 months showing sickle hemoglobin on paper electrophoresis or giving a positive sickle test, was further subjected to agar gel electrophoresis to distinguish the sickle trait from sickle cell anemia (Fessas 1965).

Experience suggests that the small amount of sickle hemoglobin present during the first month of life may make this simple screening program unreliable during this period. Although the participants in this survey were of necessity selected by a hospital attendance it is of interest that the expected 1 per cent of sickle cell anemia cases were detected.

The increase in the percentage of sickle trait carriers between the first and second year of life is significant. Malaria is a rare cause of death in this area, whereas death from respiratory and gastrointestinal infection is common. This suggests that the sickle carrier state may give some increased resistance to generalized infection as well as to malignant malaria.

<table>
<thead>
<tr>
<th>Age Examined</th>
<th>Hemoglobin Genotypes</th>
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<tr>
<td></td>
<td>AA.</td>
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<tr>
<td>0-1</td>
<td>865</td>
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<tr>
<td>1-2</td>
<td>574</td>
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<td>2-3</td>
<td>177</td>
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<tr>
<td>3-4</td>
<td>91</td>
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<td>Totals:</td>
<td>1707</td>
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REFERENCES


HEMATOLOGY OF DELETION OF β 6 OR 7 GLUTAMIC ACID. L. N. Went, Leiden, Holland.

The description of the structural analysis that was performed on this Hb Leiden has been published elsewhere (W. W. W. de Jong, L. N. Went and L. F. Bernini, Nature 220:788–790, 1968). This Hb which was found in 3 generations of a Dutch family had not given rise to any evident clinical signs and symptoms but for the index case. This woman gave a history of 3 periods of jaundice, the last one accompanied by a severe fall in Hb to 6 Gm./100 ml. All 4 carriers of this abnormal hemoglobin, which has an electrophoretic mobility like Hb S at pH 8.6 and which is present in an amount of 30–35 per cent, do have reticulocytosis which varies between 3 and 6 per cent. Their hemoglobin level is within the normal range for their age and sex, giving evidence for the existence of a well compensated hemolytic anemia. Incubation of the red cells with acetylphenylhydrazine gives rise to a marked increase in the number of Heinz bodies per cell as compared to normal blood. The sickling test is negative, the morphology of the red cells is slightly abnormal (anisocytosis, some poikilocytosis and increased basophil stippling). The methemoglobin content of the blood was normal and did not increase above the level of a control specimen after incubation up to 24 hours. The heat stability of the abnormal hemoglobin was grossly abnormal. The percentage of the abnormal fraction did not differ between old and young red cells that were obtained by high speed centrifugation. The results of in vivo and in vitro labelling of the blood with 51Cr and 59Fe (by Dr. Eernisse) will be reported separately.


During 1953–1958 a survey of the bone changes in the hemoglobinopathies was made in Jamaica. This was compared with the conditions seen in Nigeria during 1961–62 and again with a survey carried out in Jamaica in 1968. It has been found that the pattern of the skeletal changes has altered considerably both in their frequency and their severity.
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Hyperplastic changes radiologically and clinically are becoming less frequent and the morbidity they cause, less important. The gross skull changes seen in West Africa and to a lesser extent, in Jamaica before 1958 are now a rarity. The vertebral changes are unusual and the stature of many patients with the homogygous condition may be within normal limits.

Bone infarcts are also less common and the dactylitis so common in West Africa is hardly seen. Bone infarcts still affect long bones in childhood and adolescence but less frequently become infected with either the usual pyogenic organisms or the salmonellae. This may be due to the antibiotic cover these patients receive routinely.

Hip changes complicating the heterogygous sickle cell hemoglobin C and thalassemia states are seen and cause a certain amount of disability. The homogygous SS patient may present with avascular necrosis of the head of the femur, usually in early adolescence. Cases where infarcts have been discovered in the head of the humerus have been seen.

It is believed that the general improvement in the pattern of the hemoglobinopathies results from improved nutrition, the absence of malaria and comparative lack of other parasites which themselves used to result in severe hyperplastic anemia.

LABORATORY STUDIES IN SICKLE CELL ANEMIA. P. F. Milner and G. R. Serjeant.

(1) Sickle cell anemia and glucose-6-phosphate dehydrogenase deficiency

Following the reports of Lewis and Hathorn1 and Lewis, Kay and Hathorn2 that there is a high incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency, as evidenced by Brewer's test,3 in patients homozygous for Hb S, it was decided to examine the rate of methemoglobin reduction in the presence of methylene blue in Jamaican subjects with sickle cell disease. The method used was based on that of Beutler and Baluda4 whereby, after treatment of washed red cells with nitrite, the cells were rewashed in buffer to remove nitrite from the system, a standard concentration of methylene blue was added and the mixture incubated at 37 C. At suitable intervals, aliquots were removed, the cells lysed and their methemoglobin content measured. The results were plotted against time and, both for normal and for blood containing various amounts of Hb S, the methemoglobin behavior followed zero order kinetics. The methemoglobin reduction rate (MMR) taken from the slope was independent of the age of the blood for up to two weeks storage in ACDI. The rate was usually faster than normal in sickle cell anemia and a good correlation was found between MMR and units of enzyme activity as assayed with an NADP, glucose-6-phosphate, 6-phosphogluconate system.5 For G6PD deficient red cells from patients with normal hemoglobin, sickle cell trait or sickle cell anemia, first order kinetics in the MMR were associated with low assay activity. It was concluded that Hb S did not interfere with methemoglobin reduction. However, in view of the possible difficulty of interpreting the Brewer’s test in anemic sickle cell blood, 110 male patients homozygous for Hb S were examined by the methylene blue tests of Sass.6 In 30 of these an assay was also done and six examples of unequivocal G6PD deficiency were detected. In a further three the Sass test was positive but the assay result was in the normal of high normal range. As the G6PD activity in sickle cell anemias was usually more than twice normal, we considered that they were probably also G6PD deficient. In a survey of sickle cell trait males 9 out of 62 (14.5 per cent) were G6PD deficient, whereas for males with normal hemoglobin 61 out of 458 (13.3 per cent) were deficient. It was concluded that no double selection operated for Hb S and G6PD deficiency in Jamaica such as had been described in Ghana.

(2) Enzyme content of irreversibly sickled cells (ISCs)

Blood with a reduced plasmacrit was centrifuged at about 120 X 10³ X g. in a spinoce model L ultracentrifuge and the packed cells divided into a top layer containing reticulocytes, a middle layer, and a bottom layer which consisted almost entirely of deformed cells (ISCs). Red cells from these layers were resuspended in their own plasma, oxygenated, smears made and aliquots used for determination of MCH, MCHC and MCV and cell size profile using a Coulter model F red cell counter. Typically MCH remained constant in the layers but MCHC was increased and MCV decreased in the bottom layer as compared
Comparison between ISCs and red cell survival did not fall on this line having both a high reticulocyte percentage so that assays of 0.659. Patients with a palpable spleen correlation of Hb 55 tends to remain constant in time but to vary greatly from case to case. It would thus appear that whatever determines the formation of ISCs in the blood the rapid removal of these cells largely determines the degree of hemolysis.

(3) Correlation of ISCs with red cell survival as measured with $^{51}$Cr

The percentage of ISCs in the blood of a patient with Hb SS tends to remain constant in time but to vary greatly from case to case. A good correlation was found between $^{51}$Cr TCrT and percentage of ISC with a coefficient of 0.659. Patients with a palpable spleen did not fall on this line having both a low ISC count and shorter TCrT in most instances. It would thus appear that whatever determines the formation of ISCs in the blood the rapid removal of these cells largely determines the degree of hemolysis.

(4) Comparison between CrT 1/2 and $^{59}$Fe turnover

$^{59}$Fe turnover was measured in six patients and varied from 5.6 to 2.6 times normal. The shorter the CrT 1/2 the greater the iron turnover except in folate deficiency (one case) where a degree of ineffective erythropoiesis was associated with a high iron turnover and CrT 1/2 of seven days and in renal failure (one case) where a CrT 1/2 of 5 days was associated with an iron turnover only 3.8 times normal. In patients with a CrT 1/2 of 9–13 days the iron turnover was only 2.5-3.5 times normal, in spite of venous hematocrit levels of 20-30 per cent. Erythropoiesis seems to be balanced against hemolysis at a low hematocrit level in sickle cell disease as compared to hereditary spherocytosis. This could be accounted for by the large shift to the right in the oxygen dissociation curve of Hb SS blood as compared to HS subjects. If this is true the patient with sickle cell anemia cannot be considered to have responded maximally to his degree of anemia because his degree of anemia is compatible with adequate oxygenation of the tissues. This is perhaps fortunate because higher hematocrit levels are associated with a greater incidence of thromboembolic phenomena such as priapism, pulmonary infarction and painful crises.

REFERENCES
6. Sass

THE NATURAL HISTORY OF SICKLE CELL ANEMIA IN ADULTS. Graham Serjeant.

The experience of the adult sickle cell anemia clinic at the University Hospital of the West Indies was reviewed. The clinic has been built up gradually over a period of 4 years. Four clinics operated at hospitals in the country and a mobile clinical unit enhance the completeness of follow up and family studies. At present, 300 adults with homozygous SS disease are followed of which 160 are women and 140 are men. Eighty-four patients are over the age of 30 years. The commonest complication was leg ulceration in 63.3 per cent starting most commonly between the ages of 10–20 years. Leg ulcers commencing for the first time after the age of 25 years were uncommon. Admissions for painful crises occurred in 30 per cent of the group but this became less common with increasing age. This is not solely accounted for by the diminishing numbers in the older age group. For example in the 15–19 age group, there were admissions in 27 out of 70 patients (38.5 per cent), in 20–24 age group 26 out of 67 (38.8 per cent), in 25–29 age group 12 out of 42 (28.6 per cent) in 30–34 age group 5 out of 33 (15.2 per cent), in 35–39 age group 1 out of 30 (3.3 per cent) and there were no admissions in the 21 patients over the age of 40 years.
Pneumonia or pulmonary embolism accounted for admissions in 25 per cent of male patients and 28 per cent of female patients. It involved the lower lobes in 75 per cent cases. Priapism occurred in 26 patients (18.6 per cent). Aplastic crises were recorded in 23 patients (7.7 per cent) and 21 were affected under the age of 13 years. There were 4 instances of multiple cases in one family at the same time. Duodenal ulceration occurred in 20 patients (6.7 per cent). In male patients over 25 years old there were 15 cases of duodenal ulceration in a group of 64 (23.4 per cent). Attacks of osteomyelitis or avascular necrosis occurred in 38 (14.2 per cent) and epistaxis in 16 (5.3 per cent). Tetanus occurred on 11 occasions in 10 patients. Growth and maturity commenced at 15 years or later in 74 per cent of women. The persistence of splenomegaly into adult life is more frequent than previously described. It fell from 38 per cent in the 12–14 age group, 19 per cent in 15–19 age group to 9.5 per cent in 20–24 age group. It did not decrease further being palpable in 11 of 122 cases over the age of 25 years (9.0 per cent). The tendency of leg ulcers to heal, the decrease in painful crises and the infrequency of aplastic crises, with increasing age all contribute to an amelioration of the condition as the patient gets older. The natural history of the disease in the West Indies is more benign than traditional descriptions of this disease from elsewhere.

**Pregnancy Complicated by Sickle Cell Disease. M. F. Anderson.**

Two hundred and two pregnancies occurred in our patients with sickle cell disease, Hb SS—116, Hb SC—70, SThal.—16. Maternal mortality, due mainly to painful crises, "pneumonia," and increased anemia was highest in Hb SS, 54 per cent; in Hb SC, 26 per cent.

Painful bone and joint crises had a peak incidence at thirty-six weeks gestation, were rare in labor, and uncomplicated by acute sequestration, or bone marrow and fat emboli. Management was with analgesics. Heparin and exchange blood transfusion were not given.

In severe abdominal crises, the incidence of stillbirths, fetal anoxia and premature labor was high.

Maternal and fetal risks were high in cases with "pneumonia," especially those with cardiac failure or severe anemia.

Thirty per cent of our patients had hemoglobin contents below the normal range for sickle cell disease, due to deficiency of iron or folic acid, inadequate replacement of blood loss at delivery, "pneumonia" or renal disease, and aplastic crisis. Slow transfusions of packed cells, with folic acid or iron when necessary were given. Extremely low hemoglobin levels requiring exchange transfusions have not been seen, probably due to the absence of malaria, and the rarity of megaloblastic anemia in this population.

Folic acid was given prophylactically during pregnancy, and preliminary investigations suggest that supplementary oral iron is also necessary.

The incidence of prematurity, fetal anoxia and perinatal mortality was highest in Hb SS, being 33.8 per cent, 33.0 per cent, and 15.0 per cent respectively, and was two to three times higher than in Hb SC. Perinatal mortality was more commonly associated with severe anemia, abdominal crises, pneumonia or severe toxemia.

There was one maternal death in 187 births, due to secondary postpartum hemorrhage. Our lower maternal mortality may be partly due to the more benign course of sickle cell disease in Jamaica.

A STUDY OF LUNG FUNCTION IN SICKLE CELL ANEMIA. G. J. Miller.

Acute pulmonary episodes are a common complication of sickle cell anemia, although their nature often remains uncertain. It is generally believed that the sickling phenomenon is the basis of most of these episodes, and they are frequently termed embolic or thrombotic. In addition there might be a progressive occlusion of the pulmonary vascular bed leading to a pulmonary diffusion defect. We have investigated this possibility in thirteen patients so far.

Patients answered a respiratory questionnaire, and then their ventilatory capacity was measured. Total lung volume and its subdivisions were estimated by the closed circuit Helium dilution method, and the transfer factor (T1), diffusion capacity of the lung membrane (Dm), and the pulmonary capillary blood volume (V) were measured by the method due to Roughton and Forster.
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Eight patients admitted to previous acute respiratory disease, but in no case was there any suggestion of chronic respiratory disease. There was no evidence of uneven ventilation, chronic obstructive airways disease or restrictive lung disease. In four cases the transfer factor was normal and in two cases it was reduced solely because of the anemia, for $D_m$ and $V_a$ were normal. The transfer factor was moderately reduced in three patients, and markedly reduced in four due to a reduction in $D_m$. Pulmonary capillary blood volumes were normal in all cases.

The explanation for this finding favored by the author, and which is in closest agreement with current concepts of sickle cell lung disease, is that the low $D_m$ in seven of the thirteen patients is the result of a reduction in the area of the pulmonary blood/gas interface by widespread pulmonary capillary occlusion. The normal $V_a$ suggests dilatation of the surviving capillary bed. There are alternative explanations however, including altered hemodynamics of the pulmonary vasculature, capillary membrane edema, and the possibility that calculated values for the rate of reaction of carbon monoxide with hemoglobin (θ) are inappropriate for Hb S.


It has been claimed that patients with sickle cell anemia in “painful crises” often have a severe metabolic acidosis. Urinary acidification was therefore investigated in 14 adult patients with sickle cell anemia to determine if there is any defect of renal function which might affect their ability to excrete an acid load and hence provide an explanation for the tendency to develop acidosis. The patients were given a standard load of ammonium chloride orally (0.1 Gm./Kg. body weight) and the urine pH and hydrogen ion excretion measured on an hourly basis for 8 hours afterwards. The results showed that these patients cannot excrete an acid load as efficiently as normal controls. The lowest mean urine pH obtained was 5.3 compared with 4.8 for the normal controls. Their excretion of hydrogen ion was also significantly reduced. These findings, in the absence of any systemic acidosis, means that these patients must have the syndrome of incomplete renal tubular acidosis.

In an attempt to elucidate the nature of this defect, the response to intravenous sodium sulfate, which is the most potent stimulus of urinary acidification known, was studied in 8 patients and 4 normal controls. The results showed a normal response to sodium sulfate. Since this test investigates mainly distal tubular function, it is concluded that the defect does not lie in the distal tubule.

We have also investigated the acid-base status of these patients during “painful crises” because despite reports from elsewhere, we do not use alkalis for the routine treatment of patients in “painful crises.” Our results in 10 patients in “painful crises” showed no evidence of metabolic acidosis. These results support our view that alkalis do little to abort or alter in any way the acute painful episodes of our patients with sickle cell anemia.

UNSTABLE HEMOGLOBIN HEREDITARY ANEMIA. H. Lehmann.

The nature of the hemoglobin molecule was discussed. A particular type of hemolytic anemia is the unstable hemoglobin hereditary anemia. The usual cause is a mutation which interferes with the maintenance of the structure of the molecule and might either cause instability of one (or rather two) of the hemoglobin sub-units, α or β, or it might cause a failure to form dimers from two different sub-units or tetramers from two dimers respectively. With Hemoglobin E, it can be demonstrated that the abnormal β chain does not combine with the normal α chain as efficiently as does the normal β chain. This causes the Hemoglobin E proportion to fall when Hemoglobin E trait is found in combination with α thalassemia. In Hemoglobin E homozygotes, inclusion bodies can be observed which seem to consist of α chains. These inclusion bodies could be the cause of the hemolytic anemia arising in such homozygotes. It was pointed out that Hemoglobin S and Hemoglobin C behave similar to Hemoglobin E in as much as that in the heterozygote they amount to rather less than 50 per cent of the normal hemoglobin and that the proportion falls further if the trait for Hemoglobin S or Hemoglobin C is combined with α thalassemia. It is suggested
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that the hemolytic process in homozygotes for the sickle cell hemoglobin and for Hemoglobin C may at least in part be due to the formation of α chain inclusion bodies which would cause hemolysis in exactly the same manner as inclusion bodies cause hemolysis in unstable hemoglobin diseases.

The Sudden Rise of Platelets and Reticulocytes in Different Sickle Cell Crises. A. van der Sar.

Hematologic studies performed during recovery phases of aplastic sickle cell crisis led to the hypothesis that the occurrence of possible periodical cyclic changes in platelets and reticulocytes could be the explanation of an aplastic sickle cell crisis. Subsequent serial platelet and reticulocyte counts obtained during the recovery phase of an aplastic sickle cell crisis revealed a typical platelet and reticulocyte crisis followed by a renewed platelet crisis with an elevated plateau formation. The reticulocyte level remained normal.

"Pain crisis" due to bone marrow and bone infarction showed a platelet and reticulocyte crisis quite similar to the recovery phase of aplastic sickle cell crisis. Pulmonary infarctions secondary to bone marrow infarctions were marked by a platelet crisis without a reticulocyte crisis.

Primary pulmonary infarctive processes displayed similar hematologic findings, platelet crisis with a long-standing elevated platelet plateau without a reticulocyte crisis. The plotted data suggested a shortened life span of platelets during different sickle cell crises. It was postulated that platelet crisis with plateau formation may be due to a peripheral demand for platelets as a consequence of platelet aggregation "en masse" in the formation of sickle cell "thrombosis," a so-called "coagulation-consumption effect."

Reference


The Irreversibly Sickled Cell. William C. Mentzer, Charles S. August, and David G. Nathan.

Sickle-cell anemia (Hb SS disease) provides opportunities to study in man the significance of a variability from cell to cell in the relative proportions of similar proteins which coexist within individual members of a cell population. Work from this laboratory has shown that net synthesis of fetal hemoglobin (Hb F) is least in erythroid precursors destined to become irreversibly sickled cells (ISC), and that these morphologically obvious deviants suffer preferential destruction. Cell content of total hemoglobin is constant from cell to cell; hence absolute amounts of Hb S and Hb F vary reciprocally within any one Hb SS cell population.

Structural deformation of erythrocyte membranes by a filamentous alignment of deoxygenated Hb S molecules is presumably responsible for reversible sickling, but hemoglobin within ISC can be either filamentous (sickled) or structureless (non-sickled). Further definition of ISC and non-ISC by transmission electron microscopy has revealed an aberrant appearance of ISC membranes suggesting structural damage. A characteristically tight longitudinal ordering of filaments in deoxygenated ISC probably mimics filament arrangement during postulated periods of sequestration which lead to irreversible cell distortion.

It appears likely from these studies that low net synthesis of Hb F during early maturation of an erythroid precursor is responsible for this ultimate chain of events: (a) facilitated sickling; (b) increased tendency to sequestration; (c) membrane damage and consequent irreversible deformation; and (d) cell death.

Androgens stimulate erythropoiesis; in sickle hemoglobin syndromes an additional effect—reduction of sickling—has recently been proposed. We treated 2 female SS disease patients with IM testosterone enanthate for 2–3 months and observed an elevation of 255 cc. and 460 cc. in their respective total RBC volumes with a concomitant rise in hemoglobin concentration. Work capacity (measured by ergometry) and sense of well being improved. Therapy did not alter the 51Cr RBC life span or the sickling proclivities of the patient's erythrocytes. The latter was assessed by enumeration of irreversibly sickled cells, by the Na metabisulfite test, and by studies of K+ loss from ouabainized RBC exposed to varying O2 tensions. Total fetal hemoglobin, synthesis of gamma chains by reticulocytes, and the usual heterogeneous distribution of HbF in erythrocytes were unchanged by therapy. Whole blood viscosity, measured in a rotational viscom...
eter, and RBC deformability, measured by a millipore filtration, were unimproved. A third patient, a male treated with oral oxymetha-

lood cell volume. Unlike our previous pa-
tients, his RBC lifespan (51Cr) was slightly
lengthened by therapy and his irreversibly
sickled cells fell from 35 per cent to 5 per
cent. Fetal hemoglobin and viscosity were
not altered by therapy. In this patient, an-
drogens may have influenced the sickling
phenomenon but if so the mechanism re-
mains obscure.

Complications of androgen therapy in-
clude fluid retention and priapism which led
to cessation of therapy after 2 weeks in an
8 year old male. Reversible virilization of
females was also observed.

Further study of the effect of androgens
in sickle cell syndromes with particular ref-
erence to possible effects on the rate of
sickling are in progress.

PULMONARY LESIONS IN SICKLE CELL ANEMIA. L. W. Diggs.

A study of autopsy material from 72 pa-
tients with sickle cell diseases other than
the sickle cell trait, including 62 with sickle
cell anemia revealed multiple pulmonary
lesions attributable to the hereditary hemo-
globin abnormality including sickled eryth-
rocytes, engorgement of blood vessels with
sickled cells, proteinaceous material in al-
veoli, emboli in pulmonary arteries, infarcts,
serofibrinous pleural effusion and pleural
adhesions.

Emboli consist of entangled mats of
sickled erythrocytes and fragments of intra-
vascular clots performed in systemic veins.
Emboli may also consist of globules of fat
and bits of cellular marrow and trabecular
bone from areas of ischemic infarction of
bones. Organization of emboli is charac-
terized by ingrowth of fibrocytes, recanal-
zation and plaques. A study of fresh emboli
reveals no anatomical evidence of primary
thrombi in pulmonary arteries.

Emboli and infarcts were observed in one
of 32 children with sickle cell anemia less
than 10 years of age. Fresh and organized
pulmonary emboli were demonstrable in 18
of 30 patients with sickle cell anemia and
gross infarcts in 12. Pulmonary embolism
and infarction constitute a major risk during
pregnancy and during and following de-
livery.

Studies of pulmonary function reveal evi-

dence of a progressive decrease in arterial
oxygen tension, an increase in alveolar-ar-
terial oxygen gradient, a shift in oxygen-
hemoglobin dissociation curve to the right,
exercise intolerance and a decrease in total
lung capacity.

Blood smears of patients with pulmonary
emboli reveal evidence of cell membrane
injury characterized by blister, helmet,
dome, triangular and thorn cells as well as
spherical cells and small, dark and irregu-
larly shaped cells.

Pulmonary emboli in patients with sickle
cell diseases are frequently misdiagnosed as
pneumonia. Differential diagnosis is aided
by examination of smears of sputum for
acellular reaction, blood and bacteria; ex-
amination of shape of red cells in stained
blood smears and a differential count of
leukocytes.

CLINICAL FEATURES OF S.C.A. IN JAMAICAN
CHILDREN. R. Gray.

One hundred and twenty-two children
aged 0–12 years with hemoglobin SS or
hemoglobin S-thalassemia are reviewed in
detail.

Sixty-three per cent of children had their
first symptom of disease before the age of 2
years. Pain is the predominant first symp-
tom. Another common and diagnostic first
symptom is swelling of the hands and feet
due to underlying aseptic necrosis of bone.

Using Jamaican standards, physical
growth does not appear to be retarded in
this group of children. Splenomegaly is a
common clinical finding and the incidence
of splenomegaly falls off with increasing of
age from a figure of 68 per cent of children
examined at age 6–12 to 30 per cent of
those examined at 12 years. Thirteen pa-
tients developed hypersplenism hepatomeg-
aly is an invariable finding but is a common
clinical feature in normal Jamaican children.

Clinical and radiologic cardiomegaly is
frequent, as is L.V.H. on electrocardiog-
raphy. Average Hb levels fall in the range
7–10 Gm. per cent in 75 per cent of pa-
tients. Average bilirubin levels fall in the
range 2–4 mg. per cent in 66 per cent
cases.

Painful febrile crisis is the commonest
complication of the disease in children.
Pains involve limbs, abdomen and back. The
severity and frequency of pain varies from
postmortem and septal lymphatics associated with males ranging in age from 7 months to 76 at mortem.

The clinical course is variable and diagnosis. Some patients, chiefly involving the R.L.L. Other vascular accidents (3), severe obstruction jaundice (7), cerebral vascular accidents (3), osteomyelitis (3), and priapism (1).

Two hundred and seventy-five children with S.C.A. have attended U.C.H. at some time since 1952. Thirty deaths have been recorded among these. In 7 cases no anatomic cause for death could be found at autopsy.

S.C.A. in children is a serious disease but does not necessarily carry a hopeless prognosis. The clinical course is variable and many children lead a relatively trouble free life.

POSTMORTEM APPEARANCES OF THE LUNGS IN 28 PATIENTS WITH HOMOZYGOUS SICKLE CELL DISEASE. W. W. Whimster.

The macroscopic descriptions and histologic sections from the lungs taken at postmortem at the University Hospital of the West Indies between 1956 and 1969 were reviewed. There were 15 males and 13 females ranging in age from 7 months to 76 years. Electrophoresis had shown an SS hemoglobin pattern in all cases.

There were no pathognomonic features. Inflammation in the form of pneumonia, lung abscess, or empyema was present in 15 cases; but infarction or arterial thrombi were seen in only 7, 2 of them under 10 years of age. Iron-laden macrophages were seen in a case of leukemia and in one of aplastic anemia, and also in a case of mitral stenosis. Their absence in the other patients suggested that heart failure was not a common complication. However in a postmortem conducted personally the only pathologic abnormality was a marked distension of pleural and septal lymphatics associated with perivascular edema histologically. The latter was seen in 10 other patients and resembled that seen by Heath and Hicken (Thorax 15:54, 1960) in patients with mitral stenosis.

In 8 of the 11 cases under 10 years and 4 of the 17 cases over 10 years no anatomic cause of death could be found at postmortem.

FOLIC ACID STUDIES IN S.C.A. IN WEST AFRICA. E. J. Watson-Williams.

This work was done during the years 1957–1962 from the Department of Pathology of the University College Ibadan, Nigeria and the main results have been published.1,2 Of 875 cases of homozygous hemoglobin S disease fifty were found to have megaloblastic erythropoiesis. Laboratory results indicated folic acid deficiency without vitamin B12 deficiency. Among the patients with folic acid deficiency were several with clinical malnutrition and the whole group showed a lower mean height and weight than other patients of the same age with sickle cell anemia. Following treatment with folic acid weight gain and improved well being was striking. These results led to detailed investigation of four young adults (3 female and 1 male) with delayed puberty and growth and sickle cell anemia. Although erythropoiesis was normoblastic they all showed biochemical evidence of folic acid deficiency. Treatment with folic acid was associated with a spurt in growth and the occurrence of puberty within 12 months but no change in hemoglobin concentration. These four patients all had red cell survival times (51Cr T½ 2.8–6.0 days) within the lowest range found in other patients with sickle-cell anemia (51Cr T½ 3–12 days). These results support the hypothesis that folic acid requirements are proportional to erythropoietic activity. It is recommended that all patients with sickle cell anemia have prophylactic doses of folic acid throughout life.

REFERENCES

QUANTITATIVE MEASUREMENTS OF OXYGEN SATURATION AND SICKLING OF INCUBATED BLOOD. R. A. Lewis.

A method is described in which blood is placed in heparinized microhematocrit tubes which are kept on a conkey shaker in an incubator. At two hour intervals tubes
ABSTRACTS: SICKLE CELL CONFERENCE

SICKLING AND HEMATURIA. D. W. Atkinson.

The incidence of positive sickling in cases of macroscopic hematuria was 29.3 per cent instead of the expected 11 per cent. One hundred fifteen cases of hematuria and a positive sickle test have been investigated. Thirty-four had good cause for their hematuria (10 had tumors), 7 were incompletely investigated and 74 had no cause other than a positive sickle test to account for their hematuria.

Investigations

Hb Electrophoresis AS 66, SC2, SS1, S+F1, not known 4. IVP good visualisation (hyposthenuria not a problem) 4 only showed papillary necrosis, 4 filling defects due to clot. Cystoscopy revealed bleeding L ureter 18, R 12, Bil 4. Three kidneys removed —no micro or macroscopic cause for bleeding.

Clinical Features

Ages 14–70. 43 male and 31 female. Hematuria characteristically profuse, total and painless. Ten per cent only had pain and twenty per cent only passed clots. Thirty-eight patients bled from 1–14 days, only 8 had repeat bleeds. Twenty-nine bled for 1–6 months and 7 bled for more than a year. Uncommon for severe anemia to develop. Only 8 required transfusion. Trauma was associated with 7 cases and the amount and duration of the bleeding was out of proportion to that normally associated with trauma.

Management was conservative. E.C.A. therapy will control the bleeding temporarily.
If it is profuse, the etiology remains quite unknown. The author leans to the theory that there is some abnormality of urinary fibrinolysis to account for the bleeding.

**Oxygen Dissociation Studies of Red Cells in Sickle Cell Disease.** E. R. Huehns and A. J. Bellingham.

A method of determining the oxygen dissociation properties of hemoglobin in red cells suspended in isotonic phosphate buffers was described. The results confirm that intracellular hemoglobin in sickle cell disease has a significantly lower oxygen affinity than the hemoglobin in normal cells. In sickle cell trait an almost normal curve was obtained, while in sickle cell β-thalassemia with 10 per cent Hb-A an intermediate oxygen affinity was obtained. It is suggested that the low oxygen affinity found in sickle cell disease is due to a constraint on the molecule by the two new intermolecular bonds, which cause the polymer formation during the sickling process, tending to keep the molecule in the low affinity (deoxy) conformation. This hypothesis implies that the propensity to sickle and the change of oxygen affinity in the cells are the same process. In order to explain the larger than expected effect of Hb-A on the sickling process and on oxygen affinity in sickle cell-thalassemia disease and sickle cell trait it is suggested that these break up the sickle hemoglobin polymer not only by the presence of normal molecules, but also by the presence of the hybrid species of Hb-αβαββ. The effect of Hb-F and Hb-A2 would also be enhanced by the presence of the hybrid species Hb-αβαγγ and Hb-αβασσ in the cell. It is well-known that sickling will occur in cells containing another hemoglobin as well as Hb-S but only at relatively low oxygen tensions. The occurrence of sickling in these conditions implies that long polymers of sickle hemoglobin molecules have formed and that molecular sorting out has taken place in the cell. The ease with which this can take place may vary with different normal and abnormal hemoglobins present in the cell and as a result the severity of the various mixed syndromes (Hb-S+C, Hb-E+S and Hb-O+S as well as sickle cell trait) may be very different although the proportion of Hb-S in the cells may be very similar.

As has already been pointed out it is likely that the change in oxygen affinity in sickle cell disease is related to the propensity of the cells to sickle. It is therefore suggested that drugs which increase the oxygen affinity of sickle cells might be of use in treatment of this disease, and that measurement of changes of oxygen affinity in red cells might be a useful in vitro screening method of possible substances. However, until suitable drugs for long-term prophylaxis in this disease are found better methods for the treatment of painful crises are needed. One approach to this problem may be the use of hyperbaric oxygen as this may allow enough diffusion of oxygen into the stagnant sickled cells in the affected area for these to unsickle and thus terminate the crisis. Our own experience suggests that this may be a promising form of treatment. Another problem in the clinical management of the disease is the occurrence of anemia. Recent work in our laboratory has shown that in chronic hemolysis there is a relationship between the degree of anemia and the red cell oxygen affinity implying that the oxygen delivery capacity is very similar in all cases. This relationship also applies to the anemia seen in sickle cell disease and accounts for the well-known clinical observations that the anemia usually presents very little problem in this disease and only needs treatment when the postulated equilibrium is upset. Some of the problems mentioned above are discussed in detail elsewhere (see references).


Abstracts from the First International Sickle Cell Anaemia Conference

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